NITROGEN REQUIREMENTS OF SOUTHERN PLAINS SMALL MAMMALS: DIETARY NICHE, LIFE HISTORY, AND ENVIRONMENTAL IMPLICATIONS

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For my late grandfathers, Ted Parsons, Jr. and Gene Paxton. Your memories are always with me, and the way in which you lived your lives serves as a constant inspiration. I wish you could both be here to witness the completion of this step in my life's journey, though I know you are ever present in spirit.

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NOMENCLATURE

ADF	acid detergent fiber
ad libitum	free-choice feeding
AND	apparent nitrogen digestibility
BM	body mass
BV	biological value
CP	crude protein
DDMI	digestible dry matter intake
DMD	dry matter digestibility
DMI	dry matter intake
EUN	endogenous urinary nitrogen
MFN	metabolic fecal nitrogen
NDF	neutral detergent fiber
SNK test	Student-Newman-Keuls' multiple-range test of sample means
TND	true nitrogen digestibility
TOBEC	total body electrical conductivity

INTRODUCTION

Human activity has precipitated significant changes to ecosystems all over the world. In recent years, climatic alterations (e.g., global warming and El Niño) have been the subject of much public debate and controversy. However, other well-documented environmental phenomena have been linked directly to anthropogenic causes and shown definite effects on habitat structure and biodiversity. One of these is the modification of global element cycles, including carbon, sulfur, phosphorus and nitrogen (Vitousek 1994). Nitrogen is one of the most important constituents of all life forms. It is an integral component of protein molecules, where the nitrogen-containing amine group maintains molecular integrity by linking amino acids through peptide bonds (Cheeke 1991; Hill et al. 1993). Nitrogen also occupies a key position in the aromatic ring structure of nucleic acids (Murray et al. 1996). As such, nitrogen is indispensable for the transmission of genetic information and for a variety of enzymatic reactions, structural and transport proteins, and other physiological functions.

Nitrogen cycling through the environment is altered principally through agricultural activity (e.g., fertilization, legume crops) and combustion of fossil fuels. In the past 20 years, rates of human-induced nitrogen fixation have climbed to a higher level than those of all natural processes combined. Furthermore, release of this element from nitrogen sinks through burning, land-clearing, and drainage of wetlands introduces even more nitrogenous compounds into atmospheric or aquatic circulation (Vitousek 1994).

Consequences of soil nitrification have been studied extensively in plant communities. In general, nitrogen-saturated forests experience decreased productivity

and higher mortality (especially among conifer populations; Vitousek 1994; Fenn et al. 1998), whereas grassland systems experience the opposite trend. Nitrogen enrichment of prairies and oldfield communities led to an increase in primary productivity and overall biomass and a shift in species composition toward more annuals, fewer perennials, more nitrogen-demanding species, and fewer plants that are adapted to low nutrient availability (Grant et al. 1977; Tilman 1987; Hall et al. 1991; Vitousek 1994). A universal decrease in species richness has been reported, both within communities and among them, as nitrification homogenizes the environment (Carson and Barrett 1988; Vitousek 1994). Additionally, nitrogen supplementation may alter succession patterns in plant communities (Tilman 1987; Carson and Barrett 1988).

Effects of excess environmental nitrogen are manifest at all trophic levels, as plants are replaced by species that tend to produce more litter and altered plant composition predisposes dead tissues to more rapid decomposition (Tilman 1987; Carson and Barrett 1988; Vitousek 1994; Fenn et al. 1998). Thus, the decomposer community is impacted and nitrogen cycling is further accelerated. At the same time, nitrogen-fixing symbionts (e.g., mycorrhizal fungi) are no longer needed by plants and are reduced in number (Vitousek 1994).

Given their role as primary consumers, small mammals may be particularly responsive to alterations in the availability of this nutrient and resulting vegetation modifications. Species-removal studies have shown that interspecific interactions or competition were of secondary importance to demographic shifts in sympatric mixed-species populations; seasonal alterations in height and density of vegetation were responsible for more variation in small-mammal assemblages (Cameron 1977; Cameron

and Kincaid 1982; Kincaid and Cameron 1982a; Turner and Grant 1987). Investigations into the effect of grassland treatments such as grazing and nutrient application have shown similar community shifts in response to changes in vegetation structure and composition (Grant et al. 1982; Hall et al. 1991). Treatment of irrigated experimental plots with supplemental nitrogen induced such a change in vegetation, as shortgrass ecosystems were converted to stands of tallgrass and the small-mammal assemblage was transformed to include herbivorous microtines that were formerly absent (Grant et al. 1977).

Protein supplementation may evoke changes in demography and distribution in cotton rat populations by altering cost-benefit tradeoffs. Eshelman and Cameron (1996) induced utilization of low-cover plots with high risk of predation through the addition of supplemental feeds. The same result may be achieved through nitrification of the soil, as the protein quality of monocots on supplemented plots increases. Therefore, long before the consequences of nitrogen supplementation, in terms of vegetation restructuring, are observed, changes in nutritional composition of vegetation may result in altered habitat use. A trend toward invasion of habitat types that were previously unavailable to this species, because the costs of colonization were too high, could occur. As with plants, soil nitrogen supplementation may change successional patterns in mammalian assemblages and shift biomass density and utilization of other nutrients, particularly energy (Grant et al. 1977).

Primary consumers such as rodents perpetuate habitat alterations while they are subject to them. One of the most significant activities is the translocation of soil from below the ground to the surface (Grant and French 1980). Because the soil is the most

important nitrogen storage pool (Fenn et al. 1998), soil movements may dramatically alter nutrient availability to the rest of the ecosystem (Tilman 1983; Inouye et al. 1987). In addition to the change in surface organic matter caused by activities such as digging and burrowing, small mammals also introduce more readily decomposed materials into the soil through the deposition of feces, urine, and clipped vegetation. Thus, significant modifications to the decomposer community may occur (Grant and French 1980; Vitousek 1994).

The effect of small mammals on their habitat is not limited to a single trophic level. Herbivores, including rodents, may have direct impacts on the producer community itself through a number of mechanisms. Soil translocation, as well as urine and fecal deposition, may facilitate germination and growth of plants by providing needed nutrients and organic matter and by reducing soil density (Grant and French 1980; Gessaman and MacMahon 1984). Small mammals aid in seed and pollen dispersal in many ways, including passive (i.e., external) transport, fruit consumption, and caching of seeds or nuts (Gessaman and MacMahon 1984). Selective or preferential herbivory may alter the species composition of the plant community or the genetics of species within the community. Herbivory itself may alter plant physiology, growth rates, and nutrient utilization (Gessaman and MacMahon 1984; McNaughton et al. 1997). In this way, modifications in small-mammal assemblages caused by vegetative changes may reflect back upon primary production, thereby amplifying the effect of the original nitrogen input (Vitousek 1994).

Within the field of animal nutrition, nitrogen is known as a dietary essential nutrient, second only to carbon in necessary ingestion (White 1993). Repeated studies on

a wide range of species have shown that a lack of sufficient dietary nitrogen may cause life-threatening pathologies. These include anorexia, reduced serum protein, anemia, hepatic lipidosis, edema, and reduced enzyme and hormone synthesis. Sublethal deficiencies may cause physiological or developmental abnormalities such as inefficient feed conversion, diminished milk production, low birth weight, and reduced growth (Pond et al. 1995).

Nitrogen nutrition also is important to the ecology of free-ranging populations. White (1978, 1993) and Mattson (1980) postulated that nitrogen is the most frequently limiting nutrient in terrestrial ecosystems. Subsequent evidence from populations in radically disparate habitats (Vitousek 1994), including those of the koala (*Phascolarctos cinereus*; Degabriele 1981, 1983) and cotton rat (*Sigmodon hispidus*; Eshelman and Cameron 1996; Schetter et al. 1998), supports these theories. The level or quality of protein in the diet of wild animals influences important population parameters such as age at first estrus, fecundity, survival, growth and recruitment (Cole and Batzli 1979; Batzli 1985; Cameron and McClure 1988; Hall et al. 1991; White 1993; Vitousek 1994; Lochmiller et al. 1995; Cameron and Eshelman 1996; Eshelman and Cameron 1996; McAdam and Millar 1999). Changes in nutrient availability may cause shifts in sex ratios among small mammals (at prenatal as well as postnatal stages), which could dramatically affect reproductive performance (Trivers and Willard 1973; Sikes 1995b, 1996b). Dietary protein restriction may result in impaired immune function, thereby predisposing individuals to opportunistic infection and compounding the effect of other environmental stressors (Davis et al. 1995).

Many species have developed mechanisms to detect nutritive values of potential food items and select their diets accordingly. Some vertebrates can selectively feed on the basis of protein quantity (Musten et al. 1974; Harriman 1977; Leathwood and Ashley 1983; Law 1992; Eshelman and Cameron 1996; McAdam and Miller 1999; Lewis et al. 2001) or quality (Murphy and Pearcy 1993). Some small mammals even have the ability to detect levels of protein in the diets of potential mates through olfaction and choose among competing individuals accordingly (Ferkin et al. 1997). Because nitrogen is limiting and individuals must compete for access to high-protein diets, a mate gaining access to such foods presumably has an advantage in terms of fitness.

In light of the importance of this element to physiological processes and population biology, considerable effort has been spent to quantify the dietary nitrogen requirements of a number of domestic and wild species at several life stages. Early work focused on livestock, companion animals, and laboratory animals (Smuts and Marais 1938; Du Toit and Smuts 1941; Armstrong and Mitchell 1955; Lassiter et al. 1956; Miller and Allison 1958; Slade et al. 1970; Chiou and Jordan 1973; Czajka-Narins et al. 1973; Prior et al. 1974; Cork and Harrop 1977; Papas 1977a, 1977b; Spreadbury 1978; Cheeke 1979; Harris et al. 1985; National Research Council 1995; Piechota et al. 1995). More recently, the scope of many investigators has expanded to include wild or exotic animals. In Australia and New Zealand, extensive research has been performed on marsupials (Brown and Main 1967; Barker 1968; Brown 1968; Hume 1974; Harrop and Degabriele 1976; Hume 1977; Foley et al. 1980; Wellard and Hume 1981; Chilcott and Hume 1984; Harris et al. 1985; Cork 1986; Hume 1986; Foley and Hume 1987; Smith and Green 1987; Wallis and Hume 1992; Barboza et al. 1993) and chiropterans (Herbst 1986; Steller

1986; Law 1992; Korine and Arad 1994; Delorme and Thomas 1996; Korine et al. 1996). In Europe and Africa, studies have been performed on fur-bearing animals (Glem-Hansen 1979, 1980; Jarosz and Barabasz 1988; Tauson et al. 1997) and wild ungulates (Crawford et al. 1968; Arman et al. 1975), respectively.

Nitrogen research on wild animals in North America has focused on ungulates and other game species (Ullrey et al. 1967; McEwan and Whitehead 1970; Smith et al. 1975; Holter et al. 1979; Sadleir 1980; Carl and Brown 1985; Priebe and Brown 1987; Schwartz et al. 1987; Asleson et al. 1996). Only recently has the attention of nutritional research on wild animals turned to smaller free-ranging animals. Whereas the tractable nature of the laboratory rat (*Rattus norvegicus*) and other strains of captive-bred laboratory animals have lent themselves to numerous inquiries into the nutrition and metabolism of domestic rodents (National Research Council 1995), little information is available on the nutritional needs of wild rodent species. Limited investigations have been performed on various microtines (Lynch and Keys 1968; Shenk et al. 1970; Spears and Clarke 1987; Ditchkoff et al. 1998) and reproductive requirements of the cotton rat (Randolph et al. 1995; Hellgren and Lochmiller 1997), but published data on the nitrogen requirements of North American murids are scarce.

Nutritional requirements show a surprising degree of taxonomic variation, even within the same family or among species that occupy the same ecological niche (Law 1992; Wallis and Hume 1992). Thus, extrapolations from related species or from species with similar habitats and diets prove insufficient when trying to construct accurate models of nitrogen flux in free-ranging animals. I examined the nitrogen dynamics of 3 genera of small mammal native to the southern Great Plains to estimate nitrogen flux in

this ecoregion and to parameterize models with which to predict community responses to increasing environmental nitrogen.

Species under Study

Four of the most common species in the tallgrass prairie are the hispid cotton rat, the fulvous harvest mouse (*Reithrodontomys fulvescens*), the white-footed mouse (*Peromyscus leucopus*), and the deer mouse (*P. maniculatus*, Muridae: Sigmodontinae) (Cameron and Spencer 1981; Spencer and Cameron 1982; Lackey et al. 1985; Wilson and Reeder 1993; Wilson and Ruff 1999). Because of their dominant position within the ecosystem (Cameron 1977; Kincaid and Cameron 1982a; Turner and Grant 1987; Wilson and Ruff 1999), I chose these 4 species as the subjects of my study. Although these animals occupy the same habitat, they coexist through partitioning of diet and microhabitat and broad differences in life-history strategy.

The hispid cotton rat is a medium-sized (100-225 g) rodent that ranges from northern South America through Central America and Mexico to the south-central and southeastern United States (Wilson and Ruff 1999). It is found primarily in grassland habitats, including oldfields, agricultural lands, and grassy ditches (Sealander and Heidt 1990; Wilson and Ruff 1999). The principal habitat requirement for this species is adequate overhead cover to protect from avian predators—cotton rats are rarely found in areas that lack a dense mat of protective vegetation (Turner and Grant 1987; Sealander and Heidt 1990; Eshelman and Cameron 1996; Schetter et al. 1998). It has been classified as a generalist herbivore, with the large majority of its diet composed of monocots, although seasonal shifts in diet and selective consumption of higher-quality food items (e.g., seeds, forbs, invertebrates, avian eggs) to ensure nutritional balance have

been reported (Cameron and Spencer 1981; Cameron and Kincaid 1982; Kincaid and Cameron 1982a, 1982b; Randolph et al. 1991; Doonan and Slade 1995; Eshelman and Cameron 1996).

Cotton rats show an intraannual bimodal pattern in numbers, with peaks in spring and fall. Interannually, they have regular periods of very low densities or near-extinction (often in response to climatic conditions) and occasional population explosions (Cameron 1977; Sealander and Heidt 1990; Wilson and Ruff 1999). This species may breed throughout the year but exhibits reduced reproduction in late summer and winter; females may produce several litters in a year (Cameron and Spencer 1981; Sealander and Heidt 1990; Wilson and Ruff 1999). Cotton rats also show wide variation in litter size (typically 6-9 pups/litter, range = 1-15; Cameron and Spencer 1981; Sealander and Heidt 1990; Wilson and Ruff 1999). Young are born after a 27-day gestation and are extremely precocial—they are born completely furred, open their eyes within 2 days of birth, and are weaned in just over 2 weeks (Cameron and Spencer 1981; Sealander and Heidt 1990; Wilson and Ruff 1999; J. L. Parsons, pers. obs.). Unlike the other species in my study, cotton rats reach sexual maturity (at 30-40 days of age) before attaining adult body mass (100 days of age; Cameron and Spencer 1981; Wilson and Ruff 1999). Reported life spans are as low as 2 months, and both sexes rarely survive > 9 months (Cameron and Spencer 1981); juvenile survival is typically low, with higher adult abundance on sampled plots (Cameron 1977).

The fulvous harvest mouse is the largest of its genus, typically weighing 9-20 g (Spencer and Cameron 1982; Nowak 1991; Wilson and Ruff 1999). It inhabits grassy fields and grass-brush habitats from the southern Plains region of the United States to

southern Mexico and northern Central America (Spencer and Cameron 1982; Turner and Grant 1987; Nowak 1991; Wilson and Ruff 1999). In the Great Plains, it is often found in tallgrass fields, shrubby fencerows, and mixed grass-brush areas (Sealander and Heidt 1990). Harvest mice are highly scansorial, and vertical habitat partitioning has been observed in sympatric populations of *R. fulvescens* and *S. hispidus*, with mice using vegetation above ground level to a much larger extent than rats (Cameron and Kincaid 1982; Kincaid and Cameron 1982a; Spencer and Cameron 1982; Turner and Grant 1987). Described as a granivore, the harvest mouse mainly eats seeds and green shoots, with shifts to invertebrates in spring and summer (Kincaid and Cameron 1982a; Spencer and Cameron 1982; Sealander and Heidt 1990; Stancampiano and Caire 1995). In parts of its range, use of dicots, dicot seeds and monocots during the fall and winter has been observed (Cameron and Kincaid 1982; Spencer and Cameron 1982; Wilson and Ruff 1999).

Reproduction in harvest mice is highly seasonal, with peaks in late spring and early fall (Cameron 1977; Spencer and Cameron 1982; Sealander and Heidt 1990; Wilson and Ruff 1999). Timing of reproduction for this species is very precise; females may delay breeding even when habitat quality is sufficient to support gestation and lactation (Cameron 1977). Litters are typically small (2-4 pups), and highly altricial young are born after a 21-day gestation period, becoming furred by about 5 days of age and opening their eyes by day 12 (Spencer and Cameron 1982; J. L. Parsons, pers. obs.). Offspring are weaned at 3 weeks of age and usually reach adult mass and sexual maturity at 35 and 60-90 days, respectively (Spencer and Cameron 1982; J. L. Parsons, pers. obs.). Life span in the wild is typically < 1 year, as opposed to 2-9 months for cotton rats (Cameron

1977; Spencer and Cameron 1982; Wilson and Ruff 1999). Densities of this species are also seasonally bimodal, with highest numbers in late summer and winter; however, unlike *S. hispidus*, the density of *R. fulvescens* is regulated by reproductive bouts rather than weather conditions or seasonal food availability, and harvest mouse populations lack the periodic outbreaks or near-extinction events common to cotton rats (Cameron 1977; Cameron and Kincaid 1982).

The genus *Peromyscus* is mainly represented by two species in the tallgrass prairie. The deer mouse is one of the most well-known and well-studied rodents in the United States. It is found throughout most of the United States and the southern tier of Canada, south into Baja California and central Mexico (Wilson and Ruff 1999). As a result of its wide geographic range, this species inhabits a number of habitat types and ecological niches. In the southcentral United States, it is found almost exclusively in open areas, including grassy or weedy fields, agricultural areas, and fencerows (Sealander and Heidt 1990; Wilson and Ruff 1999). Deer mice also show a wide variety of dietary habits, depending on their habitat and food availability, and are typically opportunistic omnivores. Food items include seeds and waste grain, nuts, berries, insects and other small invertebrates, fruits, vegetation, and fungi (Sealander and Heidt 1990; Nowak 1991; McAdam and Millar 1999; Wilson and Ruff 1999). In some parts of their range or during periods of reproduction and early growth, they may shift to a largely carnivorous diet (Grant et al. 1977; McAdam and Millar 1999). They also have been observed to cache seeds and nuts for winter food (Sealander and Heidt 1990).

Deer mice may breed at any time of the year, but mainly in March to November in the southern Plains (Sealander and Heidt 1990). They may have 2-4 litters/year, with a

typical litter size of 3-5; blind, naked offspring are born after a 3-week gestation and are weaned by 28 days of age (Sealander and Heidt 1990; Wilson and Ruff 1999). Females show a post-partum estrus and may reproduce in quick succession (Wilson and Ruff 1999). Adult mass is 10-30 g, sexual maturity is reached at 1 to 2 months of age, and individuals may live up to 1 year in the wild (Sealander and Heidt 1990; Wilson and Ruff 1999). Some populations show 3-4 year fluctuations in density with food abundance (Wilson and Ruff 1999).

The white-footed mouse typically exhibits a shift in habitat type from its congeneric and is most commonly found in low-elevation forests, woodlands and hedgerows, including the wooded edges of open grasslands, where it may forage into fields and croplands at night, and forested areas along watercourses (Lackey et al. 1985; Sealander and Heidt 1990; Wilson and Ruff 1999). This species is distributed throughout much of the eastern and midwestern United States and south along the Gulf Coast to the Yucatan (Lackey et al. 1985; Wilson and Ruff 1999). Like *P. maniculatus*, it is categorized as an omnivore and consumes a wide variety of food items varying by season and habitat (Sealander and Heidt 1990; Wilson and Ruff 1999). Typical foods include seeds, green vegetation, nuts, berries, fruits, insects, other invertebrates, and carrion; food caching for the winter also has been described in this species (Lackey et al. 1985; Sealander and Heidt 1990; Nowak 1991; Wilson and Ruff 1999).

White-footed mice may breed throughout the year, with reduced reproduction in late summer and peaks in spring and fall (although this species is not as strictly seasonal as *R. fulvescens*; Lackey et al. 1985; Sealander and Heidt 1990; Wilson and Ruff 1999). Four to 5 litters/year may be produced, with an average litter size of 4-6 pups/litter and a

22-day gestation (Lackey et al. 1985; Sealander and Heidt 1990; Wilson and Ruff 1999). Females of this species also experience a postpartum estrus (Lackey et al. 1985; Wilson and Ruff 1999). Like the deer mouse, offspring of white-footed mice are small (1-2 g) and altricial at birth, opening their eyes by 12 days of age and weaning by about 4 weeks (Sealander and Heidt 1990; Wilson and Ruff 1999; J. L. Parsons, pers. obs.). Adult mass (15-25 g) and sexual maturity are reached at about 40 days of age, and individuals typically live 1 year in the wild, with higher mortality in spring and early summer, but little mortality in winter (Lackey et al. 1985; Sealander and Heidt 1990; Wilson and Ruff 1999). This species (along with *P. maniculatus*) shows less variation in population density than species that share the same habitat (e.g., *Microtus* spp., *Reithrodontomys* spp.; Lackey et al. 1985).

Objectives and Predictions

The objectives of this study were to: 1) model nitrogen flux in small mammals at known nitrogen intakes, 2) examine species-specific reproductive responses to varying nitrogen intake, 3) investigate effects of dietary nitrogen on juvenile growth and development, and 4) characterize the impact of dietary niche and life-history strategy on nutritional physiology and resource allocation in small mammals.

Previously published comparisons of nitrogen requirements have shown that animals that typically consume higher levels of dietary nitrogen have a diminished physiological need to retain ingested nitrogen (Glem-Hansen and Eggum 1974; Robbins 1993). Nitrogen content of feeds also tends to be inversely proportional to fiber content. As a result, herbivores often have very complex gastrointestinal tracts to better digest plant cellulose, whereas carnivores have very simple tracts because they do not need to

process significant levels of dietary fiber. The net result is that herbivores assimilate a greater proportion of dietary nitrogen than carnivores and require less dietary nitrogen to meet physiological needs (Miller and Allison 1958; Glem-Hansen and Eggum 1974; Robbins 1993). Although these two dietary niches represent extreme levels of dietary nitrogen, the same relationship holds for animals that consume feeds of intermediate nitrogen content (e.g., omnivores, granivores, or frugivores). As such, I predicted that dietary niche would dictate nitrogen requirements at all life stages, such that herbivores (*S. hispidus*) would have the lowest dietary need for nitrogen, omnivores (*Peromyscus* spp.) would have the highest, and granivores (*R. fulvescens*) would be intermediate.

LITERATURE REVIEW

Nitrogen Requirements

The available literature on mammalian nitrogen requirements reflects a considerable degree of variation between species and between various life stages. The sugar glider (*Petaurus breviceps*), a marsupial that consumes mainly nectar, sap, and pollen, requires only 87 mg $N/kg^{0.75}$/day for maintenance (Smith and Green 1987), whereas a neonatal mink (*Mustela vision*) needs to ingest 4,500 mg $N/kg^{0.75}$/day to achieve normal growth and development (Glem-Hansen 1979, 1980). Between these values lie a wide variety of species whose degree of sensitivity to nitrogen availability depends on a number of factors, including dietary niche, habitat, energy metabolism, behavior, phylogenetic effects, and confounding influences of other ingested substances.

Because of the key role of nitrogen in many metabolic pathways, researchers must consider not only the details of protein utilization within an animal, but also the impact of the utilization of other nutrients. Many researchers (Wallis and Hume 1992; Robbins 1993; National Research Council 1995) point out that protein metabolism is not an independent process. Instead, it depends upon such dietary factors as energy content, proportion of gross energy to protein, amino acid balance, presence of secondary plant compounds (antagonists such as tannins and phenols), total intake, and bioavailability of amino acids (Karasov 1982; Wallis and Hume 1992; Robbins et al. 1987; Robbins 1993). Research on livestock in desert environments further indicates that water intake and nitrogen balance may be closely related (Degen and Kam 1991). Robbins (1993:17) expressed the opinion that an investigation of nitrogen requirements that does not take into account other nutrients "may be of little value."

Many reasonable attempts have been made to determine the nitrogen requirement of various species, despite the challenges involved (Tables 1 and 2). The most common investigations have been to determine nitrogen requirements for maintenance. These studies have revealed general trends in requirements according to the major categories of digestive and reproductive physiology. For the most part, eutherians have a greater need for maintenance nitrogen than marsupials (Hume 1999), and ruminants tend to require more dietary nitrogen (to ensure the health of ruminal microflora) than nonruminants (Chalmers et al. 1976; Mercer and Annison 1976; Robbins 1993). Species evolved to incorporate more high-quality protein into their diet would logically have a higher absolute requirement for nitrogen than those that subsist on feeds with a lower protein value. Therefore, obligate carnivores (e.g., felids, foxes and mink) require more dietary nitrogen than strict herbivores (e.g., equids and ruminants; Miller and Allison 1958; Robbins 1993). Indeed, Glem-Hansen and Eggum (1974) demonstrated 20% lower protein utilization in mink than in rats when fed identical diets over a variety of protein sources. They hypothesized that this difference was caused by increased rates of passage in mink (due to the shorter intestinal length seen in carnivore tracts) and concluded that it may explain their high protein requirement.

Studies have shown that many species that live in arid environments, as well as those that subsist on low-quality foods, have evolved a combination of physiological and behavioral adaptations for nitrogen conservation. Queensland blossom bats (*Syconycteris australis*), which subsist mainly on protein-poor nectars, compensate by consuming nitrogen-rich pollens (Law 1992). Many arid-zone species and ruminants have the ability to recycle urea from the gastrointestinal tract, thereby reducing the amount of nitrogen

Table 1.—Previously reported maintenance nitrogen requirements for various mammalian species. All data represent dietary nitrogen values unless otherwise noted.

Species	Nitrogen requirement		Reference
	$mg/kg^{0.75}/d$	% dietary N	
Marsupials			
Common brushtail possum			
(honey and fiber diet)	203		Wellard and Hume 1981
(eucalyptus diet)	560		Foley and Hume 1987; Hume 1999
Lowland ringtail possum	380		Chilcott and Hume 1984
Eastern pygmy possum			
(pollen diet)	41		van Tets 1998; van Tets and Hulbert 1999
(mealworm diet)	151		van Tets and Hulbert 1999
Bare-tailed wooly possum	176		Hume 1999
Quokka	126-245		Brown 1968
Eastern grey kangaroo	270		Foley et al. 1980
Euro	290-310		Brown and Main 1967; Brown 1968; Hume 1974; Hume 1999
Red-necked pademelon	600		Hume 1977
Parma wallaby	566		Hume 1986
Rock wallaby	222		Brown 1968
Hare wallaby	223		Brown 1968
Tammar wallaby	240-290		Hume 1977; Hume 1999
Kangaroo Island wallaby	289		Barker 1968
Eastern wallaroo	300		Foley et al. 1980; Hume 1999

Table 1 (continued).

Species	Nitrogen requirement		Reference
	mg/kg$^{0.75}$/d	% dietary N	
Marsupials (continued)			
Long-nosed potoroo	199[a]		Wallis and Hume 1992; Hume 1999
Brush-tailed bettong	199[a]		Wallis and Hume 1992; Hume 1999
Rufous rat-kangaroo	199[a]		Wallis and Hume 1992; Hume 1999
Common wombat	158		Barboza et al. 1993
Southern hairy-nosed wombat	201		Barboza et al. 1993
Greater glider	700		Foley and Hume 1987; Hume 1999
Sugar glider	87		Smith and Green 1987
Koala	283		Cork 1986
Average	**282**		
Ruminants			
Caribou/Reindeer	462		McEwan and Whitehead 1970
Elk/red deer	1327	0.64[a]	Papageorgiou 1978; Mould and Robbins 1981
Moose	627	1.09	Schwartz et al. 1987
Roe deer	440	0.88-1.60	Eisfeld 1974; Oslage and Strothmann 1988
White-tailed deer	370-766	0.93	Holter et al. 1979; Asleson et al. 1996
Nilgai antelope	514		Priebe and Brown 1987

Table 1 (continued).

Species	Nitrogen requirement		Reference
	mg/kg$^{0.75}$/d	% dietary N	
Ruminants (continued)			
Sheep			
Chios	188-195		Papas 1977a
Merino	1085		Smuts and Marais 1938
Average	**652**		
Nonruminant eutherians			
Queensland blossom bat	337		Law 1992
Grey-headed flying fox	457		Steller 1986
Short-tailed fruit bat	442		Delorme and Thomas 1996
Egyptian fruit bat	110-247	0.16	Korine and Arad 1994; Korine et al. 1996
Rhesus monkey	680		Robbins and Gavan 1966
Human			
(milk and grain diet)	1354-1476		Clark et al. 1972; Hegsted 1976
(egg protein)	212		Young et al. 1973
Horse			
Thoroughbred	946-1092		Slade et al. 1970
Pony	331		Prior et al. 1974
Collared peccary	815	1.09	Carl and Brown 1985
Domestic pig	746	1.60-1.92	Du Toit and Smuts 1941; Armstrong and Mitchell 1955
Black-tailed jackrabbit	382-521		Nagy et al. 1976

Table 1 (concluded).

Species	Nitrogen requirement		Reference
	$mg/kg^{0.75}/d$	% dietary N	
Nonruminant eutherians (continued)			
Snowshoe hare	416		Holter et al. 1974
Domestic rabbit	503		Cork and Harrop 1977
	1250		Cheeke 1979
Domestic cat	690		Miller and Allison 1958
Raccoon	120		Mugaas et al. 1985
Rock hyrax	311		Hume et al. 1980
North American porcupine	346-389		Fournier and Thomas 1997; Felicetti et al. 2000
Muskrat	1020		Campbell and MacArthur 1996
Antelope ground squirrel	263		Karasov 1982
Fat sand rat	242		Kam and Degen 1988
Laboratory rat		0.80-1.12	National Research Council 1995
Prairie vole	401	0.70	Ditchkoff et al. 1998
Meadow vole		1.28	Lynch and Keys 1968
Average			
(all nonruminants)	**551**		
(rodents)	**444**		
Hispid cotton rat	217		This study
Fulvous harvest mouse	529		This study
White-footed mouse	350		This study

[a]Truly digestible nitrogen

Table 2.—Reported mammalian nitrogen requirements for reproduction and growth.

Species	Nitrogen requirement				Reference
	Reproduction		Growth		
	$mg/kg^{0.75}$ /day	% diet N	$mg/kg^{0.75}$ /day	% diet N	
Brushtail possum			358		Harris et al. 1985
White-tailed deer					
(gain in mass)			3040	3.20	Ullrey et al. 1967; Smith et al. 1975
(antlerogenesis)			920-1060	1.58-1.62	Asleson et al. 1996
Rusa deer				2.40	Puttoo et al. 1998
Sheep	995-1707			3.84-4.16	Papas 1977b; Chiou and Jordan 1973
Domestic pig				1.92-2.56	Lassiter et al. 1956
Rhesus monkey				3.20	Ji et al. 1986
Domestic cat					
gestation		3.20			Piechota et al. 1995
lactation		4.00-4.80			
Mink			4500		Glem-Hansen 1979; 1980
Ferrets		1.52-1.68			Jarosz and Barabasz 1988
Domestic rabbit			442	2.40-2.56	Harris et al. 1985; Spreadbury 1978; Cheeke 1979
Laboratory rat		1.60-2.40		1.50-2.40	Czajka-Narins et al. 1973; National Research Council 1995

Table 2 (concluded).

Species	Nitrogen requirement				Reference
	Reproduction		Growth		
	$mg/kg^{0.75}$ /day	% diet N	$mg/kg^{0.75}$ /day	% diet N	
Common vole		3.20		1.60	Sugawara and Oki 1988
Field vole				1.28	Spears and Clarke 1987
Meadow vole				0.80-1.28	Shenk et al. 1970
Hispid cotton rat	1168	1.71-1.86			Randolph et al. 1995; Hellgren and Lochmiller 1997
		2.31		1.96	This study
Fulvous harvest mouse		1.29			This study

that they must consume (Hume et al. 1980; Kam & Degen 1988; Yahav and Chosniak 1989). Cecotrophs such as the ringtail possum can utilize low-quality proteins through hindgut fermentation. In addition to normal fecal pellets, they excrete cecal pellets, composed primarily of microbes and broken-down vegetable matter. These cecal pellets are then reingested, allowing the animal to recapture lost nitrogen (Wallis and Hume 1992; Pond et al. 1995).

Predictions of nutritional needs cannot be made on the basis of taxonomic classification or gastrointestinal morphology alone. Many researchers report disparate nitrogen requirements among closely related species (Brown 1968; Eisfeld 1974; Hume 1986; Steller 1986; Law 1992; Korine and Arad 1994, 1996). Similarly, comparisons between ecologically similar animals have failed to yield predicted results. Species from different habitats sometimes require the same levels of dietary nitrogen (Brown and Main 1967; Brown 1968; Foley et al. 1980; Wallis and Hume 1992), and species from similar habitats, with the same dietary preferences, can differ tremendously in obligate nitrogen intake (Karasov 1982; Kam and Degen 1988; Law 1992). For example, there is a 2-fold difference between requirements of the short-tailed fruit bat (*Carollia perspicillata*) and the Egyptian fruit bat (*Rousettus aegyptiacus*), even though both are of the family Megachiroptera and have similar dietary habits (Table 1). The brush-tailed bettong (*Bettongia penicillata*) lives in extremely arid regions of southern Australia, whereas the long-nosed potoroo (*Potorous tridactylus*), is a coastal species. However, both species have low dietary nitrogen requirements compared with other marsupials (Table 1).

Nitrogen requirements appear to be determined by a combination of phylogenetics, environment, diet, morphology, behavior, and physiology. Part of the

large degree of variation in nitrogen requirements may be explained by differences in basal metabolic rate (BMR). Smuts (1935) observed a close relationship between BMR and endogenous urinary nitrogen losses and developed equations to predict nitrogen losses based on metabolic rates. Research into the nitrogen requirements of marsupials has lent support to these early theories, because marsupials generally have lower BMRs than eutherians and also tend to have a lower need for nitrogen (Wallis and Hume 1992; Hume 1999).

To estimate nutrient needs for reproduction, one must first understand the physiological demands placed on the female. Points of maximum nutrient demand during reproduction occur during the last trimester of gestation, when the majority of fetal growth occurs, and during lactation. The majority of maternal nutrient requirements during these stages go toward building and maintaining tissues associated with the gravid uterus and the enlarged mammary gland (Robbins 1993).

During lactation, the maternal requirement for all nutrients, including protein, increases progressively until weaning as the milk intake of young increases with mass gain. The nutrient cost of lactation is 2-3 times that of gestation (Robbins 1993), and lactating individuals frequently show negative nutrient balances (including nitrogen), relying on body stores to supply the nutrients required for milk production. Milk composition changes throughout the lactation cycle and varies widely among species, with the lowest protein content seen in primates and equids and the highest in lagomorphs and some carnivores (Robbins 1993). Studies cited by Glem-Hansen (1979) found that variations in maternal protein intake do not affect milk composition but do affect milk

production. The same lack of relationship between dietary protein and milk composition was found in lactating collared peccaries (*Pecari tajacu*; Lochmiller 1984).

Nutrient requirements for growth, like those for reproduction, change according to stages of development. Young animals experience the greatest need for dietary nitrogen immediately after weaning and decrease their protein requirements with increasing age (Glem-Hansen 1980). These requirements correspond to the sigmoid growth curve seen in all animals, with the highest rate of growth occurring immediately after birth. It also has been reported that the protein content per unit of mass gain in the early stages of growth is lower in altricial species, although relative protein deposition increases in later stages. Precocial species, on the other hand, show a relatively constant level of protein deposition per unit gain throughout growth. This trend is seen in avian and mammalian species. Generally speaking, however, protein gain is consistently less variable than either fat or energy gain among young mammals and birds (Robbins 1993).

The available literature on nitrogen requirements for reproduction and growth shows the same gradations in values according to dietary habits and gastrointestinal morphology. Carnivores need the most dietary protein to reproduce and grow, followed by omnivores and then herbivores. Carnivores also display longer lactational periods relative to species of similar body size and phylogenetic origin (Sikes 1995a, 1996a). Among herbivores, ruminants require the most nitrogen for reproduction and growth, followed by cecal fermentors and then monogastrics.

Nitrogen Requirements in Rodents

Much of the published research on the nitrogen dynamics of rodents follows the same trends already discussed. Very few publications exist on the reproductive or growth

requirements of rodents (National Research Council 1995; Randolph et al. 1995; Hellgren and Lochmiller 1997); however, many studies have concentrated on the maintenance nitrogen dynamics of microtine species (Lynch and Keys 1968; Shenk et al. 1970; Batzli and Cole 1979; Cole and Batzli 1979; Spears and Clarke 1987; Obara and Goto 1988; Yahav and Choshniak 1989; Ditchkoff et al. 1998). Despite the close relationship among these species, disparate values have been reported, again indicating the difficulty in comparative analyses of nitrogen requirements across taxa. Batzli and Cole (1979) compared prairie voles (*Microtus ochrogaster*), California voles (*M. californicus*), and brown lemmings (*Lemmus sibericus*) and found interspecific differences in digestive efficiency, depending on each species' natural diet (forbs vs. grasses and sedges). These differences in digestibility could alter dietary nitrogen requirements. Obara and Goto (1988) reported that urea recycling, particularly from the cecum, plays a significant role in the physiology of Japanese field voles (*M. montebelli*) and is an important determinant of dietary nitrogen requirements.

Comparisons among rodents of different genera have shown a positive correlation between adaptations to xeric habitats and the ability to conserve or recycle nitrogen. Yahav and Chosniak (1989) compared the fat jird (*Meriones crassus*), a resident of the Saharo-Arabian desert, and the Levant vole (*Microtus guentheri*), found in mesic areas of the Mediterranean. They found that jirds have higher nitrogen balances than voles when fed the same diet, jirds (unlike voles) maintained the same level of nitrogen intake on lower-quality vegetation, and nitrogen digestibility and urea recycling in jirds increased on lower-quality forages.

Results of other comparisons of mesic and xeric species fall along the same lines. The muskrat (*Ondatra zibethicus*), an aquatic species, requires 4 times the dietary nitrogen of the antelope ground squirrel (*Ammospermophilus leucurus*) and the fat sand rat (*Psammomys obesus*), both desert rodents of the North American and Eurasian continents, respectively (Karasov 1982; Kam and Degen 1987; Campbell and MacArthur 1996). However, adaptations to similar habitats do not guarantee similar nitrogen requirements. The prairie vole (*Microtus ochrogaster*) and the meadow vole (*M. pennsylvanicus*), 2 closely related species, are both found in North American prairies, but the meadow vole requires twice as much dietary nitrogen (Lynch and Keys 1968; Ditchkoff et al. 1998). Species phylogeny and evolutionary history may play a role. For example, Wallis and Hume (1992) hypothesized that the low nitrogen requirement of the mesic-adapted long-nosed potoroo (*Potorous tridactylus*) may be explained by its close relationship with the now extinct broad-faced potoroo (*Potorous platyops*), an inhabitant of the Mediterranean climate of coastal Western Australia.

Nitrogen Limitation Hypothesis

White (1978, 1993) developed the nitrogen limitation hypothesis to explain relationships among population dynamics and nutritional ecology in wild animal communities. He postulated that most animal populations are not controlled through predation, as is commonly held, but rather through food shortages, which have the greatest impact on young members of the community. This phenomenon is first felt among herbivores (primary consumers) and expands to include other animals through trophic relationships. White (1978, 1993) identified the single most limiting factor for increases in population as the availability of nitrogenous food to sustain the rapid growth

and, more specifically, the rapid gain in nitrogen-containing tissues (i.e. lean body mass) in very young animals. Most young animals cannot obtain enough dietary nitrogen to support normal growth and development; therefore, population fluctuations result from variation in juvenile survival due to changes in the relative amount of available plant nitrogen.

After the publication of White's hypothesis, research on herbivorous species has found supporting evidence. Degabriele (1981, 1983) found that koalas selected vegetation with a low fiber content, which correlated with high nitrogen content in the leaves. He also found that in winter, koalas compensated for low levels of nitrogen in eucalyptus leaves by increasing intake. The phenomenon of "wasting disease" among koalas, typified by the death of animals with full stomachs, may be a result of weather-induced nitrogen deficiency. During droughts, eucalyptus trees fail to grow new leaves, forcing koalas to consume older leaves which decrease substantially in nitrogen content. Management practices that increase the growth of young, nitrogen-rich leaves result in "outbreaks" in koala populations, supporting White's predictions. Recent work by Schetter et al. (1998) also found support for this hypothesis among populations of cotton rats. They found a trend toward higher-quality protein content (as judged by essential amino acid balance) in habitats with high-density cotton rat populations. They also found higher concentrations of phenolics (protein antagonists) in the diets of animals from low-density populations.

Method and Considerations

The most common method used to determine maintenance nitrogen requirements is through a series of feeding trials known as balance studies (Hegsted 1976). This

method assumes that the body composition of an adult animal at constant body mass does not change. Therefore, nutrient intake must be equivalent to the amount of nutrient loss from the body (Hegsted 1976). Nutrient balance is computed by subtracting loss from intake, and the maintenance nutrient requirement is defined as the level at which body mass is at equilibrium (i.e., the level at which nutrient balance is 0). Usually, balance studies consist of the formulation of experimental diets that encompass a range of nutrient values below and above estimated maintenance needs (Holter et al. 1979; Wallis and Hume 1992). Each animal's intake and output are then determined through laboratory analysis, nutrient balance is graphically compared to intake, and the level of intake at which nutrient balance is 0 is found via regression.

Despite the common use of this technique to determine maintenance nitrogen requirements, many researchers have expressed misgivings over the accuracy of data obtained from such studies. Hegsted (1976) called for development of alternative methods and stated that until such methods are found, results of balance studies must be viewed with skepticism. Such suspicion stems from a series of commonly reported paradoxes.

The first problem, which Hegsted (1976) discussed in the above mentioned article, lies in the linear nature of the regression of nitrogen balance on nitrogen intake. Hegsted (1976; cf. Holter et al. 1979; Murphy 1993; Asleson et al. 1996) reported that at high levels of nitrogen intake, nitrogen balance continues to increase without displaying a plateau that one would expect. Using a regression of previously reported data on humans, Hegsted (1976) calculated that at an intake of 14 g/day (not an uncommon level of dietary protein), a young man would be expected to gain 25 kg/year. This obviously

does not happen; therefore, the method used to gather these data must not have accounted for all variables involved.

Balance studies usually are confined to measurements of nitrogen loss through feces and urine, and occasionally through hair or pelage loss. However, nitrogen also is lost through skin, sweat, nails or hooves, and respiration, all of which are very difficult to determine. Many researchers speculate that other sources of nitrogen loss may exist that have not yet been recognized. Therefore, estimates of nitrogen loss are necessarily underestimated. Similarly, intake can only be overestimated. An animal may not consume all of the food presented, but it can never consume more. These sources of bias combine to produce inflated estimates of nitrogen balance (Hegsted 1976; Asleson et al. 1996).

Asleson et al. (1996) comment on 2 other commonly observed phenomena that indicate inaccuracies in estimating requirements from balance studies. The animals in their study balanced nitrogen at a wide variety of intakes, confounding efforts to interpret the results of balance studies. They also reported that, although none of their study subjects were at a negative nitrogen balance, none of the animals gained weight. This conflicts with the basic premise of balance studies, that a positive nutrient balance indicates a positive change in body mass. Hegsted (1976), Holter et al. (1979), and Murphy (1993) also reported such inconsistencies. Hegsted (1976:310) concluded that "in spite of the undeniable logic which tells us that one ought to be able to define requirements utilizing balance techniques, the method appears to present as yet unresolvable difficulties." Asleson et al. (1996:751) countered that "despite their shortcomings, N balance trials remain the best available method for estimating N

requirements and can continue to provide useful and reasonable estimates," while warning that "researchers should be cautious in interpreting data collected from N balance studies."

Inherent inconsistencies of balance techniques aside, a number of concerns have been raised in the literature regarding the ability of laboratory trials themselves to accurately determine the true nitrogen requirements of animals and provide a reliable estimate of needs of wild species in their natural habitat. A number of factors potentially contribute to errors in measurement of nitrogen requirements and extrapolation of these requirements to the nutritional ecology of free-ranging animals.

One of the major premises of the determination of requirements in the laboratory is the assumption that mechanisms of nutrient assimilation and utilization have not been altered significantly from those found in the free-ranging animal. Ditchkoff et al. (1998) specifically mention that they used animals from pre-established laboratory colonies to prevent variation in results due to the stresses experienced by wild-caught animals. Robbins (1993:177) stated that investigators using captive animals must presume that: "(a) internal nitrogen metabolism is not altered by confinement and (b) other dietary ingredients that the free-ranging animal may encounter have either no effect or at least a predictable effect on nitrogen loss." Of course, it is impossible to simulate the natural environment within a very tightly controlled laboratory trial. However, controlled trials must be used to accumulate sound data with inferential value. Therefore, investigators should at least be aware of conditions that affect the accuracy of their interpretations and recognize the incongruities between the laboratory model and the wild ecosystem.

A number of issues have been raised regarding the ability of standard laboratory practices to accurately quantify the observed nitrogen flux during balance trials. Published literature often addresses various points at which loss of nitrogen in samples could skew results toward a lower estimate of nitrogen content. One critical point is during collection of liquid excreta (e.g., urine) from test subjects. Some evaporation of liquid and volatilization of solutes is inevitable. However, some researchers (Foley and Hume 1987; Smith and Green 1987; Law 1992) attempted to account for this loss by conducting recovery trials. They deposited measured amounts of urine onto the surface of metabolic cage collection funnels, waited a set period of time, collected the remaining urine, calculated the percent recovery (urine volume and amount of nitrogen), and corrected all experimental urine collections for that factor. Percent recovery varied widely among feeding trials (64-95%), but in some cases, the difference between deposited and recovered urine nitrogen was large.

Another potential source of inaccuracy is the process of sample preparation. One of the commonly chosen methods to prepare urine samples for nitrogen analysis is to dry samples at 50°C for a period of time, usually several days. This method allows for the determination of dry matter content in the urine, and the remaining sample is suitable for use with the Kjeldahl procedure or in an automated nitrogen analyzer (Law 1992; Ditchkoff et al. 1998). Given the volatile nature of some of the components of urine, especially ammonia, it is possible that some nitrogen may be lost through the drying process. Ditchkoff et al. (1998) tested the validity of this concern by preparing samples in duplicate, using freeze-drying and heat-drying, and then comparing results. They reported a 4.3% loss in heat-dried samples relative to freeze-dried samples. Therefore,

researchers should either freeze-dry samples or conduct recovery trials and correct all samples for percent nitrogen lost during drying.

Balance trials often make use of sample compositing to reduce the cost and time involved in sample analysis and avoid the problem of insufficient sample, as often occurs when using smaller species. Investigators save all samples collected from each animal during the length of the trial, combine them, and then take aliquots from the combined samples for analysis. Jenks et al. (1989) tested the validity of this method as a reliable assessment of fecal nitrogen by taking duplicate samples from white-tailed deer (*Odocoileus virginianus*) pellets and either compositing them or analyzing them individually. They found no statistical difference between the 2 sets of data and found that the cost of sample analysis was reduced by 94%. However, they also recommended that for composites to represent true sample means, samples should be combined on the basis of equal weight, rather than on the basis of equal volume or equal number of fecal pellets. Therefore, it seems that this method is valid and even preferred if samples are combined properly.

When conducting trials to determine nutrient requirements for tissue synthesis, either for reproduction (synthesis of maternal and fetal tissues) or for growth (production of new tissues within the young, growing animal), mass change in test subjects is often used to evaluate their performance on a given experimental diet. Robbins (1993) pointed out that measurements of mass gain in young, growing animals are often poor indicators of nutrient requirements for production. Changing body composition also is a crucial process during growth and therefore also must be quantified. On the other hand, Robbins (1993) recognized that whole body grinding is seldom an option for many investigators

and pointed out that none of the current indirect indices of body composition are precise enough for nutritional studies. He recommended isotopic water dilution as the most reliable method to study the modifications that constantly occur in young, growing animals.

Another potential problem that may affect data collected from animals under laboratory conditions is the tendency toward obesity in wild-caught animals when fed high-quality, ad libitum captive diets. Cotton rats are known to dramatically increase in body fat on laboratory diets, sometimes to levels as high as 50% gross body mass (D. P. Rafferty and T. A. Schetter, pers. comm.). Such an enormous change in energy metabolism undoubtedly will alter other key metabolic pathways, including those that direct nitrogen utilization. It may be necessary to control energy intake of this species before experimentation at the level required for body maintenance to prevent large fluctuations in body mass. Other murids, such as members of the genera *Reithrodontomys* and *Peromyscus*, appear to self-regulate their mass, and after an initial gain immediately post-capture, they will reduce in mass to some intermediate level and maintain that weight (D. P. Rafferty and R. T. Kazmaier, pers. comm.; J. L. Parsons, pers. obs.). Therefore, it seems that precautions to prevent large changes in body mass are unnecessary for these species.

MATERIALS AND METHODS

To determine nitrogen dynamics and requirements at various life phases, animals underwent a series of feeding trials under laboratory conditions. A captive research colony was formed using wild-caught individuals trapped at various sites in Oklahoma. Not all animals used for feeding trials were wild-caught; some experimental animals were the captive-born progeny of the wild individuals. All free-ranging animals were captured using Sherman live traps (7.6 x 8.9 x 22.9 cm), following standards established by the Animal Care and Use Committee of the American Society of Mammalogists (1998).

Research subjects were housed at the Laboratory Animal Resources facility at Oklahoma State University for the duration of the pretrial and experimental periods. Animals were housed individually (except during the breeding phase of the reproduction trial) and kept at 20-25°C under a 12L:12D cycle for the duration of the study. I operated under Animal Care and Use Protocol 723, Oklahoma State University. Mice were housed in 28- x 18- x 13-cm wire-topped plastic cages with corn-cob bedding, and cotton rats were housed in similar cages that were 48 x 25 x 20 cm.

All animals were allowed ≥ 2 weeks before undergoing experimentation to adjust to captivity. During that period, food intake and body mass were recorded on a regular basis to determine average daily intake and the ability to maintain weight on captive diets. When not in a feeding trial, research animals were offered water and a 23% crude protein rodent chow (Diet #5001, PMI Feeds, Inc., St. Louis, Missouri) *ad libitum*, except for cotton rats. Cotton rats were fed restricted portions, such that body mass was restricted to a maximum of 10% above capture weight (or 150 g for captive-born

individuals). A sample of the experimental diets also was offered to a random sample of the captured individuals to ensure palatability.

During the feeding trials, I used 7 isocaloric (20.2-22.2 kJ/g; Table 3) experimental diets (Zeigler Brothers, Inc., Gardners, Pennsylvania) formulated to represent a range of nitrogen levels. Each diet was formulated identically except for relative amounts of soybean meal and corn meal, which were changed to achieve diets of 6, 8, 10, 12, 14, 16 and 20 % protein (Tables 3, 4). Research has shown that amino acid deficiencies or imbalances can cause significant depressions in protein digestion and utilization, feed intake and growth rates, and that corn-soy diets often contain low levels of methionine and lysine (D'Mello 1994). As a result, diet formulations included crystalline lysine and methionine to prevent those amino acids from becoming limiting.

Maintenance Trials

To determine maintenance requirements, individuals from each species were selected to undergo a nitrogen balance trial (Hegsted 1976). Those individuals were randomly chosen such that 7 groups of 8 animals were formed, with equal representation of sex and experimental diet within each group. Balance trials consisted of a 7-day preacclimation period, a 7-day acclimation period, and a 5-day collection period. During the preacclimation period, each animal was fed its assigned experimental diet in its normal wire-topped cage. Animals were then moved to metabolic cages and fed the same experimental diet for the duration of the acclimation and collection periods. Animals were offered water and their designated experimental diet *ad libitum* for the length of the feeding trial. The 7 groups of animals underwent experimentation in a staggered fashion,

Table 3.—Proximate analysis of experimental rations on a dry matter basis, mean (S.E.).

Component	Percent crude protein in rations (dry matter basis, by formulation)						
	6%	8%	10%	12%	14%	16%	20%
Dry matter, %	93.19 (1.47)	94.14 (0.68)	94.00 (1.47)	94.79 (1.10)	94.28 (0.73)	93.16 (0.56)	94.07 (0.32)
Crude protein, %	7.32 (0.30)	9.88 (0.19)	11.74 (0.34)	14.65 (0.64)	15.39 (0.23)	17.95 (0.15)	21.78 (0.45)
Energy, kJ/g	20.26 (0.07)	20.86 (0.20)	21.86 (0.24)	21.53 (0.41)	22.17 (0.32)	22.08 (0.16)	21.22 (0.55)
Neutral detergent fiber, %	18.76 (1.12)	19.65 (0.39)	19.59 (0.58)	20.41 (0.52)	20.42 (0.32)	20.12 (0.19)	20.42 (0.29)
Acid detergent fiber, %	17.00 (0.35)	17.36 (0.39)	16.45 (1.04)	17.50 (0.46)	16.93 (0.66)	17.19 (0.47)	16.98 (0.47)
Ash, %	5.40 (0.12)	5.85 (0.11)	6.21 (0.08)	6.75 (0.21)	6.83 (0.16)	7.34 (0.15)	7.74 (0.04)

Table 4.—Formulation of experimental rations (Zeigler Brothers, Gardners, Pennsylvania), varying in crude protein content.

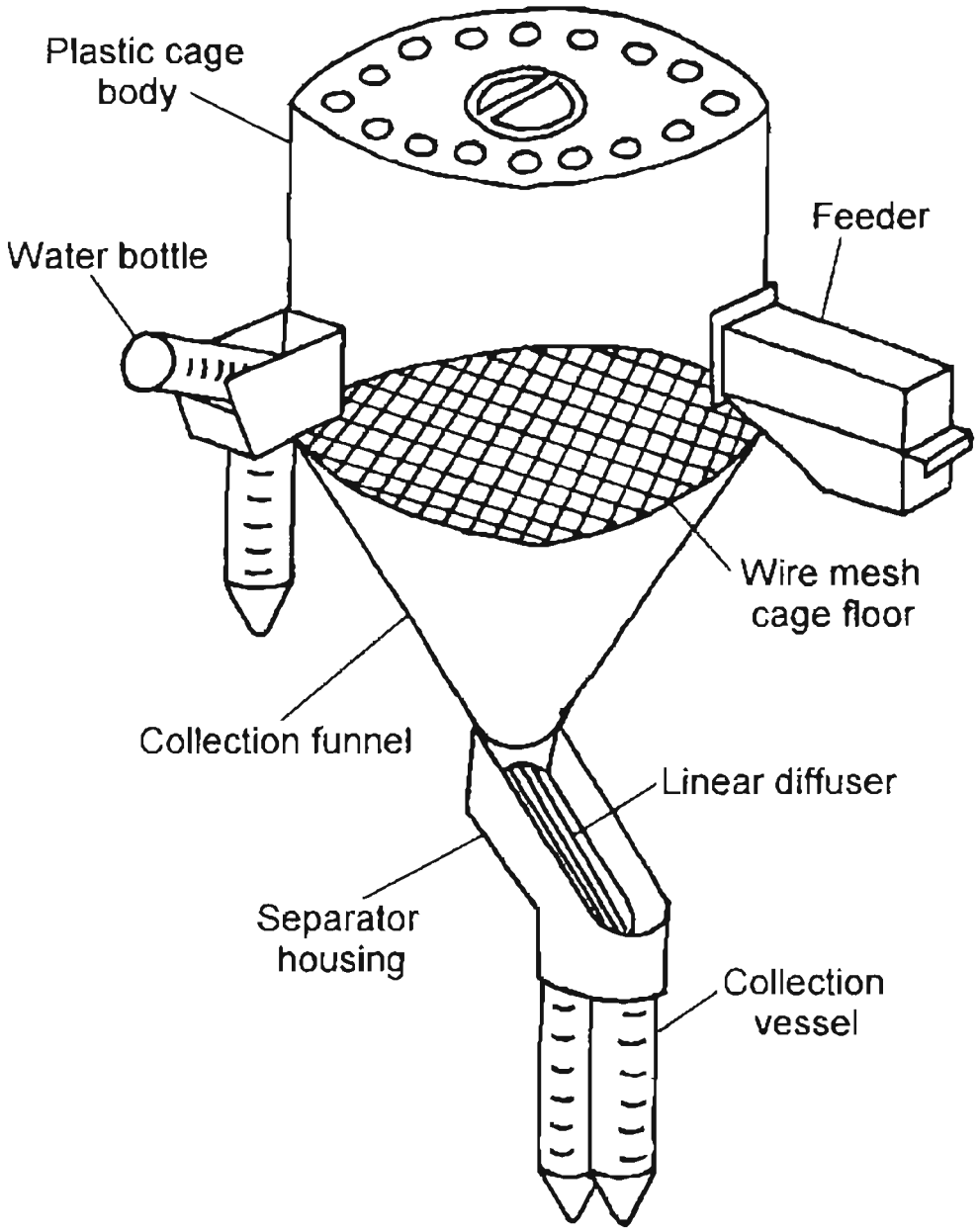
Ingredient, %	Percent crude protein in rations (dry matter basis, by formulation)						
	6%	8%	10%	12%	14%	16%	20%
Corn starch	54.88	50.82	46.77	42.71	38.66	34.61	26.50
Soybean meal	5.75	10.00	14.25	18.50	22.75	27.00	35.50
Cellulose fiber	13.50	13.50	13.50	13.50	13.50	13.50	13.50
Alfalfa	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soy oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Calcium phosphate	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Lignin sulfonate (pellet binder)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cane molasses	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine (98.5%)	1.16	1.00	0.83	0.67	0.50	0.33	----
Vitamin premix	0.25	0.25	0 25	0.25	0.25	0.25	0.25
Mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride (70%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine (99%)	0.21	0.18	0.15	0.12	0.09	0.06	----

to allow simultaneous performance of the 3 stages of the balance trial with successive groups of animals.

During acclimation and sample collection, animals were housed in plastic metabolic cages (Fig. 1; Ancare Corporation, Bellmore, New York). Those cages were self-contained units with wire mesh floors to allow feces and urine to fall into a collection funnel. The collection funnel led to a separator housing that filtered urine and feces and deposited each into its respective collection vessel. Units contained exterior compartments that dispensed food and water into the cage, thus allowing researchers access to food and water without disturbing the animal.

During the 5-day collection period, food was weighed and recorded daily to determine intake. Daily water intake (by volume) was determined and recorded. Feces were collected each day, weighed, composited in plastic bags, and frozen for later analysis. On the last day of the collection period, orts were collected, stored, and analyzed in the same way. Urine also was collected daily, measured in a graduated cylinder, composited, and frozen in polypropylene storage tubes. Urine collection tubes contained 0.5 or 2 mL of 10% H_2SO_4 (for mice and rats, respectively) to minimize volatilization of nitrogenous compounds and impede microbial growth. Collection funnels were rinsed daily with distilled water to recover any remaining urine or nitrogenous compounds. At the end of the 5-day period, funnels were more thoroughly rinsed with 10% H_2SO_4 and distilled water. Funnel washes were measured, composited, and frozen in a manner similar to urine samples. All animals were weighed at the beginning and end of the collection period to determine changes in body mass.

Figure 1. Schematic of metabolic cages (Ancare Corporation, Bellmore, New York, USA).


Plastic cage body
Feeder
Water bottle
Wire mesh cage floor
Collection funnel
Linear diffuser
Separator housing
Collection vessel

Because of problems with sample spoilage, excessive urination and the death of 1 animal, I excluded 13 cotton rats from my analyses, leaving 43 total observations. One rat also exhibited extremely low rates of dry matter intake, resulting in abnormally low fecal and urinary nitrogen excretion rates—that animal was excluded from analysis. During the course of the harvest mouse balance trials, 2 animals escaped from their metabolic cages and were removed from the feeding trial, and 1 animal died, leaving 53 observations.

Individuals belonging to the genus *Peromyscus*, particularly *P. maniculatus* and *P. leucopus*, are often difficult to distinguish from one another on the basis of physical appearance. To prevent confusion, I determined the karyotype of all individuals of this genus that underwent maintenance feeding trials (or, in the case of related individuals, ≥ 1 animal from each family unit). Animals were injected with a yeast solution 1 and 3 days before termination to induce an immune reaction. Immediately before sacrifice, they were injected with vinblastine sulfate, a mitotic inhibitor that is designed to freeze cell division at the point at which DNA molecules are most visible. Animals were then sacrificed and a sample of femoral marrow was collected, homogenized in a hypotonic solution, and concentrated. Slides of the resulting solution were prepared and dyed using Geimsa stain, then viewed via oil immersion microscopy. Species identification was accomplished by determination of the fundamental chromosomal number, according to Greenbaum and Baker (1978), Robbins and Baker (1981), and Greenbaum et al. (1994). I found that only 10 of the *Peromyscus* in balance trials were *P. maniculatus*, and because this was not an adequate sample size, deer mice were excluded from analyses. The

number of *P. leucopus* in feeding trials totaled 44, due to the death of 1 animal and 1 escapee.

Reproduction Trials

Reproductive requirements were determined during a separate feeding trial, using the same 7 experimental diets. Females of reproductive age (Table 5) were selected randomly and paired with sexually mature males for breeding. At that time, animals were offered their 23% crude protein nonexperimental diet. Females were weighed on a weekly basis to detect weight changes that corresponded to the onset of pregnancy. Each pair was housed in the same cage until it was evident that the female was pregnant (usually toward the end of the second trimester—lengths of gestation vary; Table 5). At that point, pairs were separated, and females were assigned randomly to 1 of the 7 experimental diets in a manner that ensured equal representation of all rations. I attempted to place each female on her assigned ration at the beginning of the third trimester, using changes in body mass as a guide. I chose that time period because nutritional requirements of early gestation differ little from a nonpregnant condition but increase dramatically after about 60% of the gestation period (Robbins 1993). I also sought to prevent any compounding influences caused by decreased fertility as a result of lowered nitrogen intake (Sugawara and Oki 1988). Each female stayed on her designated diet for the duration of the gestation period through weaning. Food and water were provided *ad libitum* throughout the experimental period, and cotton nesting material was provided.

Females were weighed and their intake recorded every other day before parturition. Females also were checked daily for evidence of neonates, and the first day

Table 5.—Previously reported reproductive data on the 4 species under study. (Cameron and Spencer 1981; Spencer and Cameron 1982; Lackey et al. 1985; Sealander and Heidt 1990; Hayssen et al. 1993; Nowak 1997; Wilson and Ruff 1999).

Variable	*S. hispidus*	*R. fulvescens*	*P. leucopus*	*P. maniculatus*
Litter size, range (average)	1-15 (6-9)	2-5 (3)	1-7 (4)	1-9 (3-5)
Neonatal mass, g	6-8	1.1	1.5-2	1-2
Gestation length, days	27	21-22	22-25	21-27
Time to eye opening, days	1-2	9-12	10-11	
Age at weaning, days	10-15	16-21	22-28	28
Time to reach adult mass, days	100	35	42	
Time to reach sexual maturity, days	30-40	60-90	38-44	35-50

that young were observed in the nest was treated as the date of birth. As each litter was born, the young were weighed collectively at 1 (*S. hispidus*) or 2 (*R. fulvescens* and *Peromyscus* spp.) days of age, and the number of young in each litter was recorded. Thereafter, litters were weighed every other day until each litter reached weaning age (18 days for *S. hispidus* and 21 days for *R. fulvescens* and *Peromyscus* spp.), and total litter mass and average individual mass were determined. Upon weaning, litters were removed and weighed individually, and maternal mass and intake were recorded. These juveniles were immediately entered into a feeding trial to determine nitrogen requirements for postweaning growth.

Both species of *Peromyscus* in my study failed to reproduce in sufficient numbers, so I carried out all reproductive analyses using only *S. hispidus* and *R. fulvescens*. In the process of placing animals of the latter 2 species on their assigned rations, I could not accurately estimate stage of gestation in all cases. Therefore, I excluded from analysis any individual that received experimental diets > 3 days before or after the point at which they were two-thirds of the way through gestation. Some females also refused to eat their assigned diet initially. To prevent the confounding influences of anorexia on offspring development, I excluded animals that did not return to a normal dry matter intake within a day of their placement on feeding trials. After excluding these individuals, 30 observations remained for each species.

Growth Trials

Postweaning growth requirements were determined in a feeding trial that was paired with reproductive feeding trials. Because of my difficulties obtaining sufficient numbers of young *Peromyscus*, I used only *S. hispidus* and *R. fulvescens* for growth

trials. As I weaned each litter, I weighed all individuals and determined their sex. Weanlings of both species were placed individually in the same wire-topped plastic cages (28- x 18- x 13-cm) that were used for adult mice throughout the other 2 types of feeding trials (at this age, cotton rats were too small to be housed in adult rat cages without the possibility of escape or failure to reach their food). Weanling harvest mice remained in these cages for the duration of postweaning feeding trials. Cotton rats tended to outgrow mouse-sized cages at about 3 weeks after weaning and were moved to larger wire-topped cages (48 x 25 x 20 cm) at 21 days postweaning (39 days of age).

As each litter was weaned, I randomly assigned individuals in the litter to 1 of the same 7 experimental diets used elsewhere, in such a manner that a maximum range of diets was represented in each litter. Water and experimental rations were offered *ad libitum* throughout postweaning trials. Two days after weaning and every other day thereafter, I weighed each animal and recorded its intake. Animals remained on feeding trials until 6 weeks after weaning.

Sample Preparation and Analysis

Frozen fecal samples from maintenance trials were thawed and ground in a mortar and pestle. To avoid nitrogen loss as a result of sample drying (Ditchkoff et al. 1998), 2 aliquots were taken simultaneously from each composited fecal sample. One aliquot was analyzed for nitrogen on a wet basis, whereas the other was oven-dried to constant mass (using duplicate samples) at 60°C to determine percent dry matter. All fecal nitrogen analyses were subsequently corrected for dry matter content. Samples were analyzed in duplicate for nitrogen content, either through combustion (PE-2410 Series II Nitrogen Analyzer, Perkin Elmer, Norwalk, Connecticut) or macro-Kjeldahl technique (Foss

Tecator 2400 Kjeltec Analyzer Unit, Foss North America, Eden Prairie, Minnesota), with cross-validation to preclude any bias in reported values as a result of the particular method used. Samples also were taken from each experimental ration and from previously collected orts and analyzed in the same manner for dry matter and nitrogen content. Urine and cage wash samples were thawed and analyzed as liquid samples for nitrogen content.

Experimental diets were further analyzed for nutritional content through a series of laboratory analyses. Percent ash was determined by placing samples in a muffle furnace at 500°C for 6 hours. Neutral and acid detergent fiber (NDF and ADF) were determined using an ANKOM^{200} fiber analyzer (ANKOM Technology Corporation, Fairport, New York). Mortar-ground samples were soaked in acetone for 12 hours to extract fat, then agitated in the fiber analyzer at 100°C for 60 minutes in acid detergent solution for ADF extraction. Samples were rinsed, soaked in acetone for another 3 minutes, air dried for 10 minutes, and dried in an oven at 105°C for another 2 hours. NDF extraction was carried out in the same instrument using methods adapted from Moore et al. (1987). Percent NDF and ADF were calculated as the mass of samples after extraction of soluble components divided by the original sample mass. Energy content of experimental rations was determined using standard calorimetry techniques (1425 Semimicro Bomb Calorimeter, Parr Instrument Company, Moline, Illinois). Measurements of ash, fiber, and energy content were all conducted using as-fed samples and were corrected for moisture content using previous dry matter estimates.

Data Analysis

Maintenance Requirements: General—Before analyses of balance trial data were carried out, I converted all measured values for dry matter intake, nitrogen intake, and nitrogen excretion to a metabolic body mass basis ($kg^{0.75}$) to correct for allometric effects on intake and excretion rates (Kleiber 1961; Brody 1964; Hayssen and Lacy 1985; Elgar and Harvey 1987; McNab 1988, 1989). Because compensatory intake has been reported on low-energy or low-protein diets (Shenk et al. 1970; Sinclair et al. 1982; Leathwood and Ashley 1983; Robbins 1993; Peitz et al. 1997), I tested for compensatory intake with decreasing dietary nitrogen by separately regressing dry matter intake (DMI) and digestible dry matter intake (DDMI) on percent nitrogen intake and nitrogen intake (mg), respectively. Previous studies have shown that small mammals can control their nitrogen intake by selecting food items or parts of food items that are higher in nitrogen (Shenk et al. 1970; Musten et al. 1974; Harriman 1977; Sinclair et al. 1982; Law 1992; Eshelman and Cameron 1996; Peitz et al. 1997; Lewis et al. 2001). Although the experimental rations used in this study were pelleted to ensure homogeneity of composition, I regressed ort nitrogen (%) on dietary nitrogen (%) to ensure that percent nitrogen consumed was equivalent to percent nitrogen offered. A resulting slope of 1.0, with an intercept of 0.0, would indicate a lack of selective intake. All regression analyses were carried out using simple linear regression (PROC REG; SAS Institute Inc. 2000).

Maintenance Requirements: Estimates of Nitrogen Flux—Dietary nitrogen requirements are determined by constant nitrogen losses from the body, either through feces or urine, which must be balanced with nitrogen intake (minimal amounts of nitrogen are lost through sloughed hair and skin cells, sweat, and other negligible

sources). Metabolic fecal nitrogen (MFN) is a main source of nitrogen loss and consists of sloughed cells from the intestinal lumen, digestive enzymes, undigested microbes, and other cellular debris lost from the gastrointestinal tract (Robbins 1993). It theoretically is defined as the amount of nitrogen lost in feces at a nitrogen intake of 0, but for practical reasons (e.g., decreased palatability and dry matter intake on nitrogen-free diets), it is often estimated through extrapolation from feeding trials. I estimated MFN (g/kg DMI) by regressing digestible nitrogen (g/100 g DMI) on nitrogen intake (g/100 g DMI) and multiplying the absolute value of the intercept by 10 (Bosshardt and Barnes 1946; Meyer 1956; Mould and Robbins 1981; Meyer and Karasov 1989; Asleson et al. 1996).

Endogenous urinary nitrogen (EUN), the other principal source of nitrogen loss, is the constant loss of nitrogen through the urine as a result of normal amino acid metabolism (e.g., protein turnover, gluconeogenesis) within the body (Folin 1905; Smuts 1935). Like MFN, it corresponds to nitrogen losses (mostly in the form of urea) at a nitrogen intake of 0, and also is determined experimentally through extrapolation from feeding trials. Urinary nitrogen excretion is regressed on daily nitrogen intake and EUN is calculated from the intercept of the resulting equation (Robbins 1993; Asleson et al. 1996). In addition to my determinations of MFN and EUN, I regressed fecal and urinary nitrogen (respectively) on dietary nitrogen intake to generate equations with which to predict field excretion rates. I analyzed the ratio between urinary and fecal nitrogen excretion and its relationship with dietary nitrogen to evaluate changes in the form of soil nitrogen deposition in the field. All calculations of nitrogen flux were carried out using PROC REG (SAS Institute Inc. 2000).

Maintenance Requirements: Digestive Efficiency—Efficiencies of nutrient digestion and assimilation are influenced by rates of dry matter or nutrient intake, gastrointestinal morphology, and dietary habits (Hammond and Wunder 1991; Derting and Bogue 1993; Thompson and Drobney 1996; Derting and Austin 1998; Hilton et al. 1999). To examine the effect of nitrogen intake on digestive efficiency, I performed regression analyses of dry matter digestibility (DMD) and apparent nitrogen digestibility (AND) on daily nitrogen intake using PROC REG. I also found mean true nitrogen digestibility (TND) for each species, calculated from the slope of the regression used to compute MFN (Asleson et al. 1996). I compared average DMD, AND, and TND for each species using analysis of variance (ANOVA; PROC MIXED; SAS Institute Inc. 2000).

Maintenance Requirements: Nitrogen Balance—Maintenance nitrogen requirements are most often determined through a nitrogen balance analysis. Nutrient balance is computed by subtracting loss from intake, and the maintenance nutrient requirement is defined as the level at which body mass is at equilibrium, i.e., the level at which nutrient balance is zero. I determined nitrogen requirements in this way by calculating nitrogen balance (daily urinary and fecal nitrogen excretion subtracted from nitrogen intake) for each animal and regressed nitrogen balance on daily nitrogen intake. I calculated nitrogen balance as the point at which the resulting estimated regression line intersected with a line of equation ($Y = 0$) (Hegsted 1976; Murphy 1993; Asleson et al. 1996).

All balance trial data were tested for differences between wild-caught and captive-born animals and between males and females, with two exceptions. Only 3

captive-born cotton rats and 4 captive-born white-footed mice were used for balance trials, insufficient numbers to warrant analyses of differences according to origin; therefore, only sex differences were tested for those species. Observed means were compared using 2-population *t*-tests (PROC TTEST; SAS Institute Inc. 2000) or factorial ANOVA (PROC MIXED), and comparisons in regression analyses were achieved with indicator (i.e., dummy) variables (PROC REG). If no such differences were found, data were pooled across origin or sex and analyzed as a single population. Balance trial data also were tested for differences among species using pairwise tests of probabilities for a normal distribution (Z-tests), factorial ANOVA, Student-Newman-Keuls' (SNK) multiple-range test with a 0.05 level of significance (PROC GLM; SAS Institute Inc. 2000), or indicator variables in PROC REG.

Reproductive Requirements: General—Before analyzing data from reproductive feeding trials, I tested for compensatory intake during gestation and lactation in the same manner as in maintenance trials by regressing metabolic body mass-corrected dry matter intake on dietary nitrogen. I also found the average number of days prepartum females of each species received their experimental ration (PROC MEANS; SAS Institute Inc. 2000), and performed a correlation analysis of dietary nitrogen and days on treatment prior to parturition (PROC CORR; SAS Institute Inc. 2000). A lack of correlation indicated that treatments were assigned randomly.

Reproductive Requirements: Breakpoint Analysis—Nutrient requirements for growth and reproduction are often determined through a type of regression called a breakpoint analysis or broken-line model (Robbins 1986; Nickerson et al. 1989). This is a least-squares method in which growth rates of offspring are regressed on the maternal

intake of a limiting nutrient. In principle, the resulting curve has an asymptotic shape, increasing with additional maternal intake until it reaches a point where the slope is equal to zero. One way of determining reproductive requirements is to find this asymptote, the point at which maternal diet no longer limits the growth rate of her offspring. In a breakpoint analysis, 2 straight lines are fit to this curve, with the slope of 1 line held at 0. The X-value at which these lines intersect corresponds to the aforementioned asymptote and represents the minimal maternal intake of a nutrient that maximizes growth.

I used the nonlinear regression procedure in SAS (PROC NLIN; SAS Institute Inc. 2000) to carry out this analysis, using average growth rates of individuals and litters as response variables. Research has shown that peak lactation for most mammals occurs roughly two-thirds of the way from birth until weaning (Oftedal 1984). Peak lactation also corresponds to the period of maximum maternal nutrient demand, being 2-3 times more costly than gestation (Robbins 1993). With this in mind and because I was interested in finding the minimum level of nitrogen intake to satisfy maternal needs at any given point during lactation, I used data from day 12 of lactation for *S. hispidus* and day 14 of lactation for *R. fulvescens*. The 12th day of lactation also corresponded to the time used to determine reproductive requirements for *S. hispidus* in a previous study (Randolph et al. 1995). I found the growth rate for individuals in each litter (calculated as the daily change in total litter mass divided by litter size) during the 2 days preceding and following the above-mentioned critical day for each species, and then averaged my observations over those 5 days. I also tested for the importance of maternal mass at parturition and litter size as covariates that might influence growth rate and maternal

nitrogen intake. Observed values were corrected for the influence of significant covariates.

Reproductive Requirements: Reproductive and Survival Responses to Maternal Nitrogen Intake—In addition to the breakpoint analysis, I performed a number of regression analyses on other variables associated with reproduction and lactation. To determine the degree to which dietary nitrogen affected maternal investment, I regressed total litter mass at birth and weaning, litter size, and mass lost by females during lactation on maternal nitrogen intake. All of these analyses were performed with PROC REG, using indicator variables to test for differences in performance between litters of wild-caught and captive-born females and for species differences in estimated regression equations. Finally, I performed a logistic analysis of offspring survival rates (defined as the proportion of a litter surviving to one week postweaning) to determine the level of dietary nitrogen at which successful reproduction no longer becomes possible. This analysis was performed using Graph Pad Prism, version 1.0 (Graph Pad, Inc., San Diego, California).

Postweaning Growth: General/Estimation of Requirements—Before analyzing data from growth trials, I again regressed DMI at 1, 2, and 3 weeks after weaning on percent dietary nitrogen to test for compensatory intake, using PROC REG. To determine nitrogen requirements for postweaning growth, I performed a breakpoint analysis (PROC NLIN) of weanling growth rates at week 1, 2, and 3 in the same manner as for reproductive requirements, adjusting for maternal nitrogen intake as a covariate where necessary. Because some of my study species reach their adult mass by 3 weeks

postweaning (Table 5), I performed breakpoint analyses during or before this time period to enable interspecies comparisons of growing juveniles.

Postweaning Growth: Maternal/Individual Effects on Growth—I carried out a number of multiple regression analyses to evaluate the relative effect of maternal and postweaning nitrogen intake on parameters affecting juvenile growth, maturation, and survival in the field. These analyses were conducted using PROC REG, with postweaning diet, maternal diet, the square of postweaning diet and maternal diet (respectively), and the product of postweaning diet and maternal diet as potential independent variables. Squared terms were chosen as potential variables to test for curvilinear rather than linear relationships, and the product of both diets was used to test for interactions between maternal and postweaning influences. Multiple regression models were determined through stepwise selection of independent variables, with a P-value of 0.15 as the minimum criterion for inclusion in the model.

Before conducting multiple regressions, I quantified parameters of the sigmoid growth curve using Graph Pad Prism to fit a standardized curve to each animal's changing body mass over time. Through this method, I estimated adult body mass (the point at which the growth curve reached its asymptote), time to adult mass, and maximum growth rate (calculated as the slope of linear portion of the growth curve around the inflection point; Robbins 1993) for all weanlings. I performed multiple regressions of these 3 response variables, weanling mass at 3 and 6 weeks postweaning, efficiency of nitrogen utilization at week 1, 2, and 3, and percent lean body mass at 6 weeks after weaning on dietary nitrogen. To test for evidence of compensatory growth among individuals whose mothers received low-nitrogen diets during gestation and

lactation (Wilson and Osbourn 1960; Watkins et al. 1991; Pond et al. 1995; Lepczyk et al. 1998; Lochmiller et al. 2000), I regressed mass gain during the first 3 weeks postweaning (mass at weaning subtracted from week 3 body mass) on dietary nitrogen. Data from growth trials were tested for effects of weanling sex and maternal origin using factorial ANOVAs (for comparison of mean values) or indicator values (to test for differences in response to nitrogen intake).

RESULTS

Maintenance Requirements

General

I did not find a relationship between DMI (g/kg$^{0.75}$/day) and % nitrogen in the diets of *S. hispidus* ($n = 42$, $P = 0.15$, $r^2 = 0.05$), or between DDMI (g/kg$^{0.75}$/day) and nitrogen intake (mg; $P = 0.31$, $r^2 = 0.03$). I found differences by sex in mean DMI and DDMI ($P = 0.02$ and 0.03, respectively), with males showing higher mass-specific intake rates. When I performed regression analyses using indicator variables, there was no change in the response of DMI ($P = 0.10$) or DDMI ($P = 0.08$) to nitrogen intake for either sex. Calculated separately, daily DMI and DDMI was 51.08 ± 1.60 g/kg$^{0.75}$ and 35.32 ± 1.19 g/kg$^{0.75}$ for males and 45.90 ± 1.50 g/kg$^{0.75}$ and 31.68 ± 1.12 g/kg$^{0.75}$ for females, respectively. Using species averages for DMI, DDMI, and body mass (Table 6), I estimate that the average daily DMI of adult cotton rats of 100 – 150 g would be 8.58 – 11.63 g, and average daily DDMI would be 5.93 – 8.03 g.

Because a large portion of the food offered was chewed into small pieces and dropped into the collection funnel, I was unable to analyze orts from cotton rats. DMI was calculated by separating spilled food from collected excreta (mechanically or through centrifugation) and adjusting intake calculations for spillage mass.

Balance trial data for *R. fulvescens* showed no relationships between DMI (g/kg$^{0.75}$/day) and % dietary nitrogen ($n = 53$, $P = 0.45$, $r^2 = 0.01$) or between DDMI (g/kg$^{0.75}$/day) and nitrogen intake (mg; $P = 0.87$, $r^2 < 0.001$). I found an interaction ($P = 0.05$) between sex and origin for mean DMI, with no sex differences for either captive-

Table 6.—Comparison of nutritional data from maintenance balance trials for *S. hispidus* (n = 42), *R. fulvescens* (n = 53), and *P. leucopus* (n = 44).

	Species					
	S. hispidus		*R. fulvescens*		*P. leucopus*	
Variable	$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*
Mass (g)	135.41	2.90	14.70	0.21	27.44	0.73
Dry matter intake (DMI; g/kg$^{0.75}$/d)	48.24	1.15	68.46	1.42	49.06	1.20
Digestible DMI (DDMI; g/kg$^{0.75}$/d)	33.33	0.85	42.07	1.22	32.70	0.83
Metabolic fecal nitrogen (MFN; g/kg DMI)	1.72	0.33	2.00	0.44	1.73	0.34
Endogenous urinary nitrogen (EUN; g/kg$^{0.75}$/d)	158.41	1.15	----	-----	237.34	1.22
Dry matter digestibility (DMD; %)	69.02	0.40	61.07	0.92	66.68	0.52
Apparent nitrogen digestibility (AND; %)	73.16	0.66	65.98	0.76	67.98	0.68
True nitrogen digestibility (TND; %)	81.75	1.39	75.87	1.83	77.05	1.46

born or wild-caught animals and no differences according to origin for males ($P \geq 0.10$). However, captive-born females had higher ($P = 0.04$) DMI than wild-caught females. I also found an interaction ($P = 0.04$) between sex and origin for mean DDMI, with captive-born females consuming more digestible dry matter than captive-born males ($P = 0.02$) or wild-caught females ($P = 0.08$). However, the relationship between DMI and DDMI and nitrogen intake did not change with sex or origin, either for main effects ($P \geq 0.59$) or interactions ($P \geq 0.11$). Average intake rates and body mass (Table 6) indicated that an adult harvest mouse of 12-17 g would have an average daily DMI of 2.48 – 3.22 g and an average daily DDMI of 1.53 – 1.98 g.

The regression of ort nitrogen (%) on dietary nitrogen (%) did not have a slope different than 1 or an intercept different from 0 ($n = 49$; $Y = 0.15 + 0.97X$; $P < 0.001$ for regression; $P = 0.38$ for H_0: slope = 1; $P = 0.06$ for H_0: intercept = 0; $r^2 = 0.95$). I found a marginal ($P = 0.05$) difference in this relationship between captive-born and wild-caught males (captive-born animals had a slightly smaller intercept but a higher slope) and a difference ($P = 0.01$) between captive-born males and females (males had a larger intercept and higher slope). However, none of the regressions of % ort nitrogen on % dietary nitrogen for any of these 3 groups individually had a slope different than 1 or an intercept different than 0 (all $P \geq 0.19$).

Analysis of data for *P. leucopus* indicated no significant relationships between DMI and dietary nitrogen ($n = 44$, $P = 0.52$, $r^2 = 0.01$) or DDMI and nitrogen intake ($P = 0.11$, $r^2 = 0.06$), with no effect of sex on either regression ($P \geq 0.35$) or on mean DMI or DDMI ($P \geq 0.69$). Using species averages for intake and body mass (Table 6), I calculated that an average adult of 20-30 g would consume 2.61 – 3.54 g DMI and 1.74 –

2.36 g DDMI per day. The slope for the relationship between orts and dietary nitrogen was < 1 and the intercept was > 0 ($n = 43$; $Y = 0.10 + 0.97X$; $P < 0.001$ for regression; $P = 0.02$ for H_0: slope = 1; $P = 0.004$ for H_0: intercept = 0; $r^2 = 0.99$). This regression did not differ by sex ($P = 0.30$).

Estimates of nitrogen flux

For *S. hispidus*, daily digestible nitrogen intake (g/100 g DMI) was positively related ($P < 0.001$) to dietary nitrogen (g/100 g DMI; Fig. 2; Table 7, Equation 1) and allowed the estimation of metabolic fecal nitrogen (MFN; Table 6). Regression of average daily excretion of urinary nitrogen (log mg/kg$^{0.75}$) on average daily nitrogen intake (mg/kg$^{0.75}$; $P < 0.001$; Fig. 3; Table 7, Equation 2) was used to calculate endogenous urinary nitrogen (EUN; Table 6). I found positive relationships between fecal nitrogen excretion (mg/kg$^{0.75}$/day) and % nitrogen intake ($P < 0.001$; Table 7, Equation 3) and between urinary nitrogen excretion (mg/kg$^{0.75}$/day) and % nitrogen intake ($P < 0.001$; Table 7, Equation 4).

I did not find any differences between sexes for any measure of nitrogen flux ($P \geq 0.11$), although male cotton rats nearly ($P = 0.06$) had higher EUN than females. Calculated separately, males excrete 218.67 ± 1.31 mg EUN/kg$^{0.75}$/day and females excrete 114.06 ± 1.21 mg EUN/kg$^{0.75}$/day.

Balance trial data from *R. fulvescens* indicated a positive relationship ($P < 0.001$) between digestible nitrogen intake (g/100 g DMI/day) and dietary nitrogen (g/100 g DMI/day; Fig. 2; Table 7, Equation 5) and a corresponding value of MFN (Table 6).

Table 7.—Equations for predicting nitrogen flux and nitrogen balance for cotton rats (*Sigmodon hispidus*), harvest mice (*Reithrodontomys fulvescens*), and white-footed mice (*Peromyscus leucopus*) under variable % dietary nitrogen.

Equation #	Species	Equation	Parameters of regression model		
			n	*P*	r^2
1	*S. hispidus*	g digestible N/100 g DMI/day = -0.17 + 0.82 * (g N intake/ 100 g DMI)	42	< 0.001	0.99
2	*S. hispidus*	mg urine N/kg$^{0.75}$/day = 158.41 + e$^{0.001 * (\text{mg N intake/kg\^0.75/day})}$	42	< 0.001	0.70
3	*S. hispidus*	mg fecal N/kg$^{0.75}$/day = 122.02 + 69.32 * (% N intake)	42	< 0.001	0.54
4	*S. hispidus*	mg urine N/kg$^{0.75}$/day = -101.87 + 329.32 * (% N intake)	42	< 0.001	0.79
5	*R. fulvescens*	g digestible N/100 g DMI/day = -0.20 + 0.76 * (g N intake/ 100 g DMI)	53	< 0.001	0.97
6	*R. fulvescens*	mg fecal N/kg$^{0.75}$/day = 156.56 + 154.48 * (% N intake)	53	< 0.001	0.65
7	*P. leucopus*	g digestible N/100 g DMI/day = -0.17 + 0.77 * (g N intake/ 100 g DMI)	44	< 0.001	0.98
8	*P. leucopus*	mg urine N/kg$^{0.75}$/day = 237.34 + e$^{0.001 * (\text{mg N intake/kg\^0.75/day})}$	44	< 0.001	0.33
9	*P. leucopus*	mg fecal N/kg$^{0.75}$/day = 63.16 + 123.68 * (% N intake)	42	< 0.001	0.78
10	*P. leucopus*	mg urine N/kg$^{0.75}$/day = 15.78 + 290.97 * (% N intake)	44	< 0.001	0.44

Table 7 (concluded).

Equation #	Species	Equation	Parameters of regression model		
			n	*P*	r^2
11	*S. hispidus*	(mg urine N/kg$^{0.75}$/day) / (mg fecal N/kg$^{0.75}$/day) = 1.17 + 0.001 * (mg N intake/kg$^{0.75}$/day)	42	0.001	0.23
12	*P. leucopus*	(mg urine N/kg$^{0.75}$/day) / (mg fecal N/kg$^{0.75}$/day) = 1.42 + 0.0005 * (mg N intake/kg$^{0.75}$/day)	42	0.01	0.14
13	*S. hispidus*	% DMD = 74.58 – 2.49 * (% N intake)	42	< 0.001	0.44
14	*S. hispidus*	% AND = 63.39 + 4.37 * (% N intake)	42	< 0.001	0.49
15	*R. fulvescens*	% DMD = 73.90 – 5.64 * (% N intake)	53	< 0.001	0.43
16	*R. fulvescens*	% AND = 56.42 + 4.20 * (% N intake)	53	< 0.001	0.35
17	*P. leucopus*	% DMD = 73.65 – 3.21 * (% N intake)	44	< 0.001	0.60
18	*P. leucopus*	% AND = 60.33 + 3.52 * (% N intake)	44	< 0.001	0.42
81	*S. hispidus* and *P. leucopus*	(mg urine N/kg$^{0.75}$/day) / (mg fecal N/kg$^{0.75}$/day) = 1.35 + 0.0007 * (mg N intake/kg$^{0.75}$/day)	84	< 0.001	0.17
82	*R. fulvescens*	estimated mg urine N/kg$^{0.75}$/day = 348.13 + $e^{0.001 * (\text{mg N intake/kg}^{\wedge}0.75/\text{day})}$	53	< 0.001	0.90
83	*R. fulvescens*	estimated mg urine N/kg$^{0.75}$/day = -118.05 + 623.84 * (% N intake)	53	< 0.001	0.74

Fig. 2.—Estimation of metabolic fecal nitrogen (MFN) for *S. hispidus* (n = 42, Y = -0.17 + 0.82X, $P < 0.001$, $r^2 = 0.99$), *R. fulvescens* (n = 53, Y = -0.20 + 0.76X, $P < 0.001$, $r^2 = 0.97$), and *P. leucopus* (n = 44, Y = -0.17 + 0.77X, $P < 0.001$, $r^2 = 0.98$) by examining the relationship between digestible nitrogen (g/100 g DMI) and nitrogen intake (g/100 g DMI). MFN may be estimated from the intercepts of these regressions, and TND is calculated from the slopes of the equations.

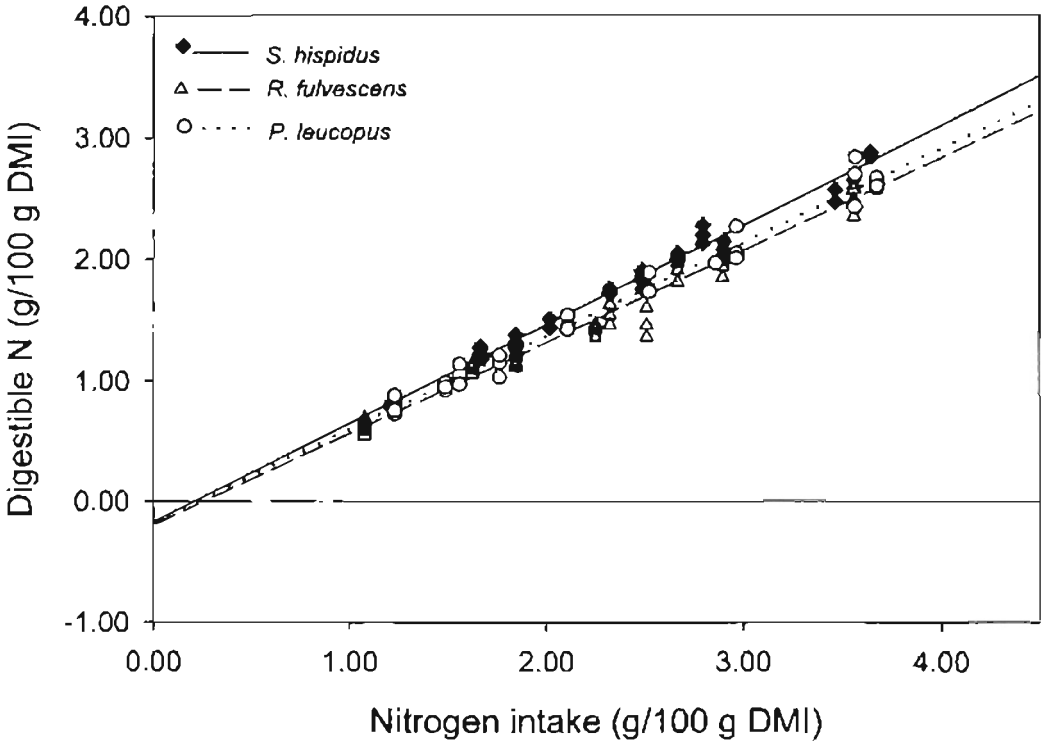
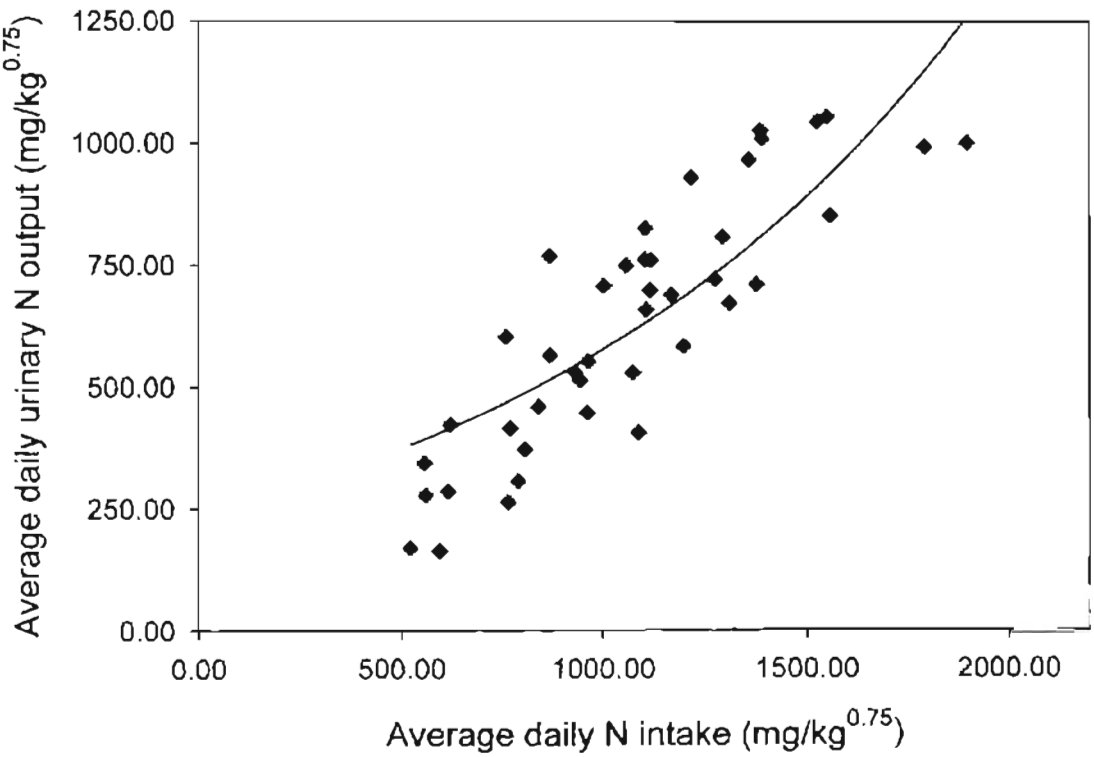

4.00
3.00
2.00
1.00
0.00
-1.00
0.00
1.00
2.00
3.00
4.00
Digestible N (g/100 g DMI)
Nitrogen intake (g/100 g DMI)
S. hispidus
R. fulvescens
P. leucopus

Fig. 3.—Estimation of endogenous urinary nitrogen (EUN) for *S. hispidus* from the relationship between average daily urinary nitrogen excretion ($mg/kg^{0.75}$) and average daily nitrogen intake ($mg/kg^{0.75}$; $n = 42$; $Y = 158.41 + e^{0.001X}$; $P < 0.001$; $r^2 = 0.70$). EUN may be estimated from the intercept of this regression (see Table 6).

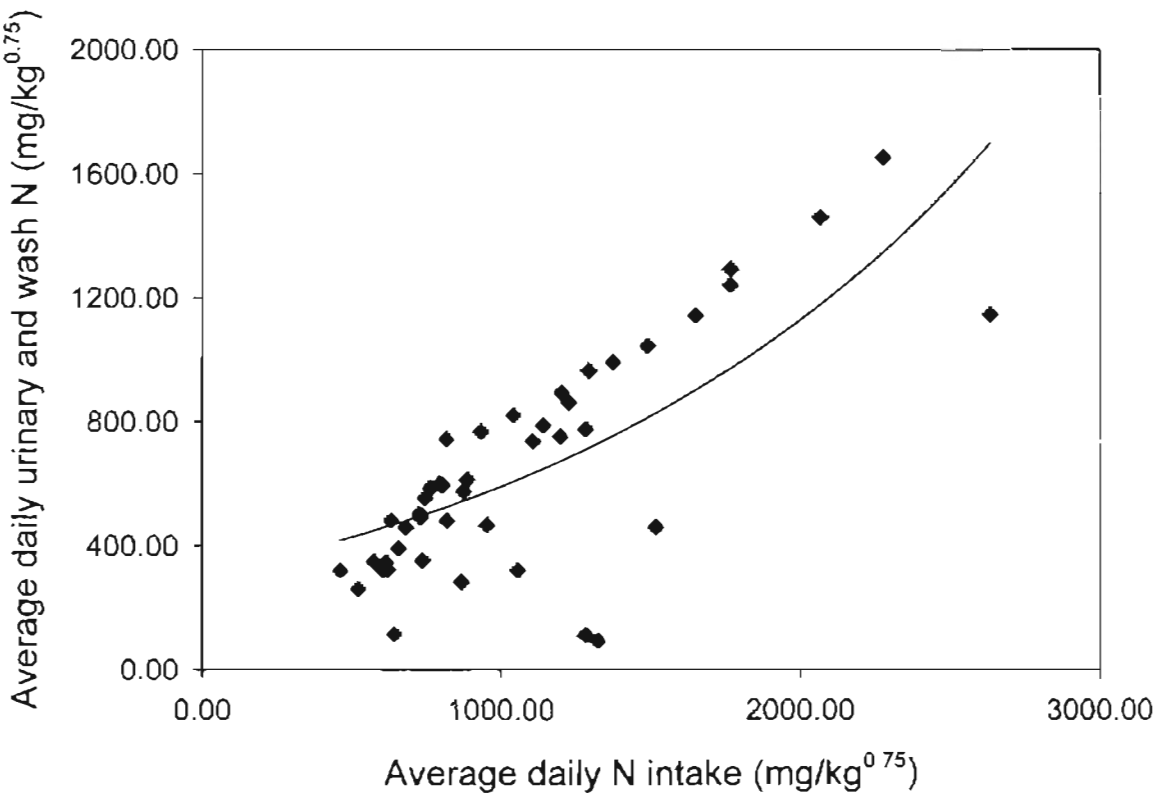

Average daily urinary N output (mg/kg$^{0.75}$)
1250.00
1000.00
750.00
500.00
250.00
0.00
0.00
500.00
1000.00
1500.00
2000.00
Average daily N intake (mg/kg$^{0.75}$)

Because of the small amount of urine excreted by *R. fulvescens*, I could not accurately collect and quantify urinary nitrogen and was therefore unable to calculate EUN or perform subsequent urine nitrogen analyses. I found a positive relationship ($P < 0.001$) between fecal nitrogen excretion (mg/kg$^{0.75}$/day) and % nitrogen intake (Table 7, Equation 6).

I did not find any origin or sex differences for any of the relationships between nitrogen excretion and nitrogen intake for harvest mice ($P \geq 0.24$), with 3 exceptions. The regression for the calculation of MFN differed by sex ($P = 0.01$) for wild-caught animals only (males had a lower intercept but a greater slope). The relationship between fecal nitrogen (mg/kg$^{0.75}$/day) and % nitrogen intake showed a sex effect ($P = 0.007$) for wild-caught mice (a smaller intercept and greater slope for males) and an effect of origin ($P = 0.03$) for females (captive-born animals had a lower intercept and higher slope). MFN was 1.72 ± 0.55 g/kg DMI for captive-born individuals, 1.73 ± 1.05 g/kg DMI for wild-caught males, and 2.37 ± 0.68 g/kg DMI for wild-caught females.

There was a positive relationship ($P < 0.001$) between digestible nitrogen (g/100 g DMI/day) and nitrogen intake (g/100 g DMI/day; Fig. 2; Table 7, Equation 7) for *P. leucopus*; MFN was estimated from this relationship (Table 6). I experienced difficulties collecting urinary nitrogen for this species and relied on cage washing to recover urine remaining on the surface of the collection funnel. Therefore, all reported values for urinary nitrogen also include nitrogen collected in cage washes. The positive relationship between urinary nitrogen excretion (log mg/kg$^{0.75}$/day) and nitrogen intake (mg/kg$^{0.75}$/day; $P < 0.001$; Fig. 4; Table 7, Equation 8) allowed calculation of EUN (Table 6). I also found a positive relationship ($P < 0.001$) between fecal nitrogen

Fig. 4.—Estimation of endogenous urinary nitrogen (EUN) for *P. leucopus* from the relationship between average daily urinary nitrogen excretion ($mg/kg^{0.75}$) and average daily nitrogen intake ($mg/kg^{0.75}$; $n = 44$; $Y = 237.34 + e^{0.001X}$; $P < 0.001$; $r^2 = 0.33$). EUN may be estimated from the intercept of this regression (see Table 6).


Average daily urinary and wash N (mg/kg$^{0.75}$)
2000.00
1600.00
1200.00
800.00
400.00
0.00
0.00
1000.00
2000.00
3000.00
Average daily N intake (mg/kg$^{0.75}$)

excretion (mg/kg$^{0.75}$/day) and % nitrogen intake (Table 7, Equation 9) and between urinary nitrogen excretion (mg/kg$^{0.75}$/day) and % nitrogen intake (Table 7, Equation 10). For the regression of fecal nitrogen on % diet nitrogen, 2 observations lay more than 2 standard deviations away from the estimated regression line; therefore these observations were not included in the analysis. There was no effect of sex on any of these 4 regressions ($P \geq 0.23$).

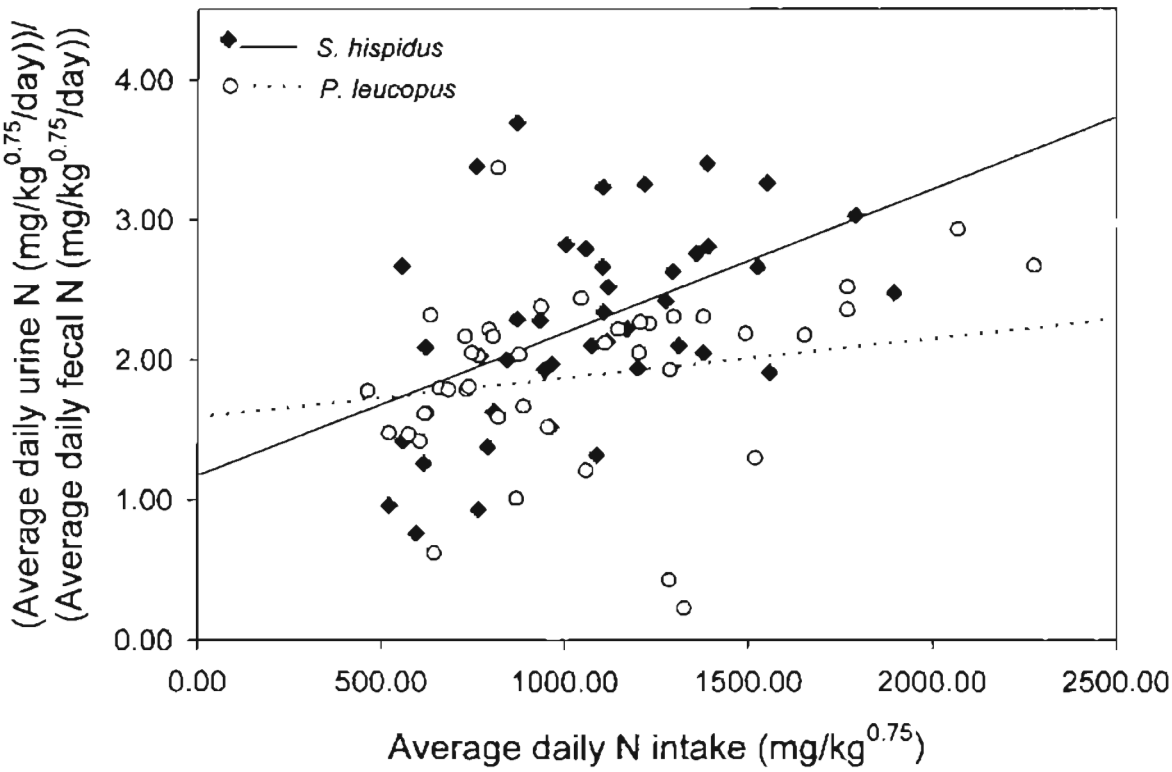
Using data on excretion rates, I regressed the ratio between daily urine and fecal nitrogen excretion (mg/kg$^{0.75}$ per mg/kg$^{0.75}$) for each animal on nitrogen intake (mg/kg$^{0.75}$/day) to model urine excretion for *R. fulvescens*. That relationship was significant for *S. hispidus* ($P = 0.001$; Fig. 5; Table 7, Equation 11) and *P. leucopus* ($P = 0.01$; Fig. 5; Table 7, Equation 12), with no sex differences for either species ($P \geq 0.08$).

Digestive Efficiency

Dry matter digestibility (DMD, %) was negatively related ($P < 0.001$) to dietary nitrogen (%) for *S. hispidus* (Table 7, Equation 13). Apparent nitrogen digestibility (AND, %) was positively ($P < 0.001$) related to % dietary nitrogen (Table 7, Equation 14). Neither the response of digestive efficiency to dietary nitrogen nor mean DMD or AND (Table 6) varied by sex ($P \geq 0.20$). Average TND (Table 6) was the slope of the regression equation of digestible nitrogen (g/100 g DMI) on dietary nitrogen (g/100 g DMI).

The relationship between DMD (%) and dietary nitrogen (%) also was negative for *R. fulvescens* ($P < 0.001$; Table 7, Equation 15), whereas the relationship between AND (%) and % nitrogen intake was positive ($P < 0.001$; Table 7, Equation 16). There

Fig. 5.—Relationship between the ratio of urinary nitrogen excretion (mg/kg$^{0.75}$/day) to fecal nitrogen excretion (mg/kg$^{0.75}$/day) and nitrogen intake (mg/kg$^{0.75}$/day) for *S. hispidus* (n = 42, Y = 1.17 + 0.001X, P = 0.001, r^2 = 0.23) and *P. leucopus* (n = 42, Y = 1.42 + 0.0005X, P = 0.01, r^2 = 0.14).


S. hispidus
P. leucopus
(Average daily urine N (mg/kg^0.75/day))/
(Average daily fecal N (mg/kg^0.75/day))
4.00
3.00
2.00
1.00
0.00
0.00
500.00
1000.00
1500.00
2000.00
2500.00
Average daily N intake (mg/kg^0.75)

were no differences in estimated regression lines or average values (Table 6) for DMD or AND according to origin ($P \geq 0.53$). However, mean DMD varied by sex ($P = 0.04$) and AND nearly varied ($P = 0.06$) by sex, with females showing higher digestive efficiencies. Similarly, the response of DMD to dietary nitrogen showed a weak sex effect ($P = 0.08$), and the regression of AND on dietary nitrogen varied by sex ($P = 0.02$). Average DMD and AND was 59.26 ± 1.46 % and 64.40 ± 1.16 % for males and 62.83 ± 1.07 % and 67.49 ± 0.92 % for females, respectively. Mean TND (Table 6) was 74.50 ± 2.30 %, 72.30 ± 4.48 %, and 79.70 ± 2.72 % for captive-born animals, wild-caught males, and wild-caught females, respectively.

When data for *P. leucopus* were analyzed, DMD (%) and dietary nitrogen (%) were negatively related ($P < 0.001$; Table 7, Equation 17), and AND (%) and dietary nitrogen (%) were positively related ($P < 0.001$; Table 7, Equation 18). Tests of estimated regression lines and average values (Table 6) according to sex showed no significant differences ($P \geq 0.74$). Average TND for this species (Table 6) was estimated from the regression used to calculate MFN.

Nitrogen Balance

The regression of nitrogen balance (mg/kg$^{0.75}$/day) on nitrogen intake (mg/kg$^{0.75}$/day) was not significant for *S. hispidus* ($n = 42$, $P = 0.998$, $r^2 < 0.001$) or *R. fulvescens* ($n = 49$, $P = 0.22$, $r^2 = 0.03$). Thus, I was unable to calculate maintenance nitrogen requirements for these species using the regression method. The same analysis of data for *P. leucopus* showed a weak significant result with poor explanatory power ($n = 43$, $P = 0.04$, $r^2 = 0.10$; Fig. 6). Dietary nitrogen requirements calculated by this method equalled 516.90 mg/kg$^{0.75}$/day.

Interspecific Comparisons

Factorial (species by sex) ANOVAs of mean DMI and DDMI showed a significant species effect ($n = 142$, $P < 0.001$; Table 6). A Student-Newman-Keuls' (SNK) multiple-range test of sample means indicated that *R. fulvescens* consumed higher amounts of dry matter than *P. leucopus* or *S. hispidus*, but the DMI of the latter 2 species did not differ. SNK analysis of mean DDMI yielded the same result.

Pairwise comparisons of observed values for MFN using *Z*-tests indicated no differences among any of the 3 species studied ($P \geq 0.61$; Table 6), although there were species effects on the relationship between fecal nitrogen (mg/100 g DMI/day) and dietary nitrogen (g/100 g; $n = 139$, $P \leq 0.01$; Fig. 2), with the most shallow slope for *R. fulvescens*, followed by *P. leucopus* and then *S. hispidus*. Estimated EUN was greater ($Z = 41.09$, $P < 0.001$; Table 6) for *P. leucopus* than *S. hispidus*, but the relationship between urine nitrogen (mg/kg$^{0.75}$/day) and dietary nitrogen (mg/kg$^{0.75}$/day) did not differ by species ($n = 86$, $P = 0.16$). There were differences among all 3 species in the relationship between fecal nitrogen excretion (mg/kg$^{0.75}$/day) and % nitrogen intake ($n = 137$, $P < 0.001$; Fig. 7), with the lowest intercept for *P. leucopus*, followed by *S. hispidus* and *R. fulvescens*, and the most shallow slope for cotton rats, followed by *P. leucopus* and *R. fulvescens*. There was no difference between *S. hispidus* and *P. leucopus* in the relationship between urinary nitrogen excretion (mg/kg$^{0.75}$/day) and % dietary nitrogen ($n = 86$, $P = 0.64$; Fig. 8).

Fig. 6.—Relationship between nitrogen balance (mg/kg$^{0.75}$/day, including cage wash data) and nitrogen intake (mg/kg$^{0.75}$/day) for *P. leucopus* (n = 43, Y = -86.79 + 0.17X, P = 0.04, r^2 = 0.10).

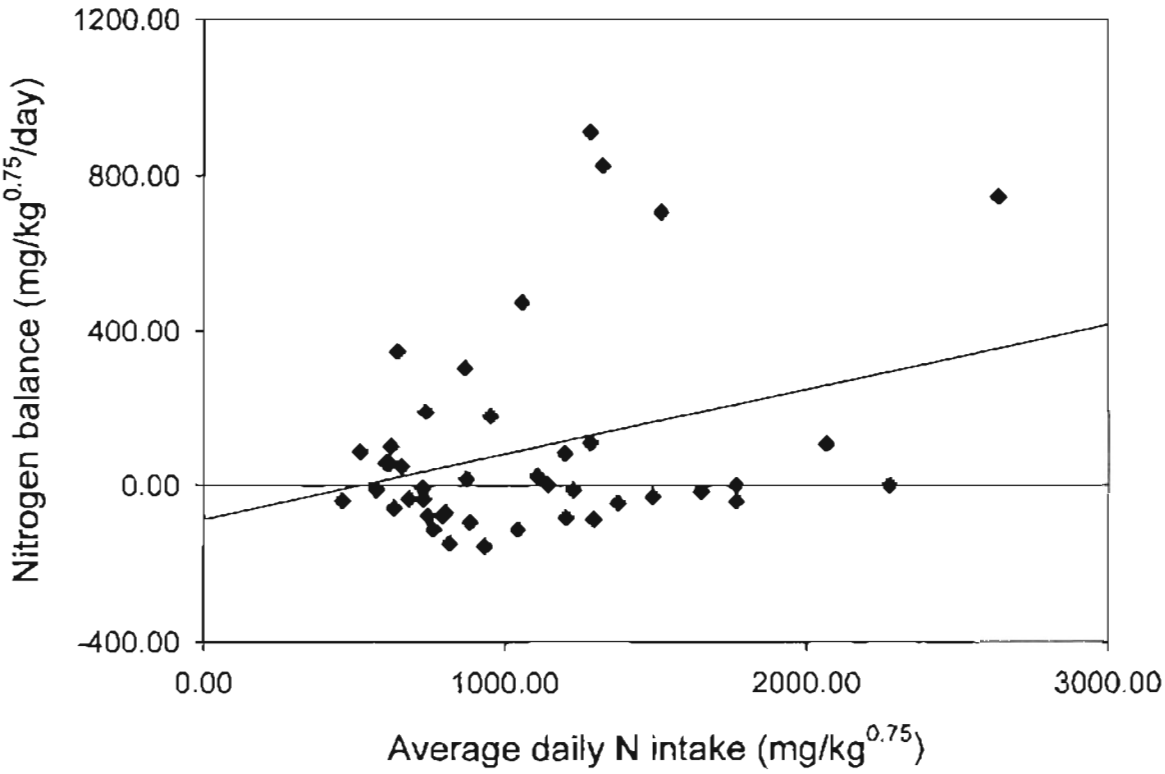

1200.00
800.00
400.00
0.00
-400.00
0.00
1000.00
2000.00
3000.00
Nitrogen balance (mg/kg$^{0.75}$/day)
Average daily N intake (mg/kg$^{0.75}$)

Fig. 7.—Comparison of estimated regression lines between *S. hispidus* (n = 42, Y = 122.02 + 69.32X, $P < 0.001$, $r^2 = 0.54$), *R. fulvescens* (n = 53, Y = 156.56 + 154.48X, $P < 0.001$, $r^2 = 0.65$), and *P. leucopus* (n = 42, Y = 63.16 + 123.68X, $P < 0.001$, $r^2 = 0.78$) for the relationship between fecal nitrogen excretion ($mg/kg^{0.75}/day$) and nitrogen intake. The open squares represent extreme values for *S. hispidus* and *P. leucopus* that were excluded from the regression analysis.

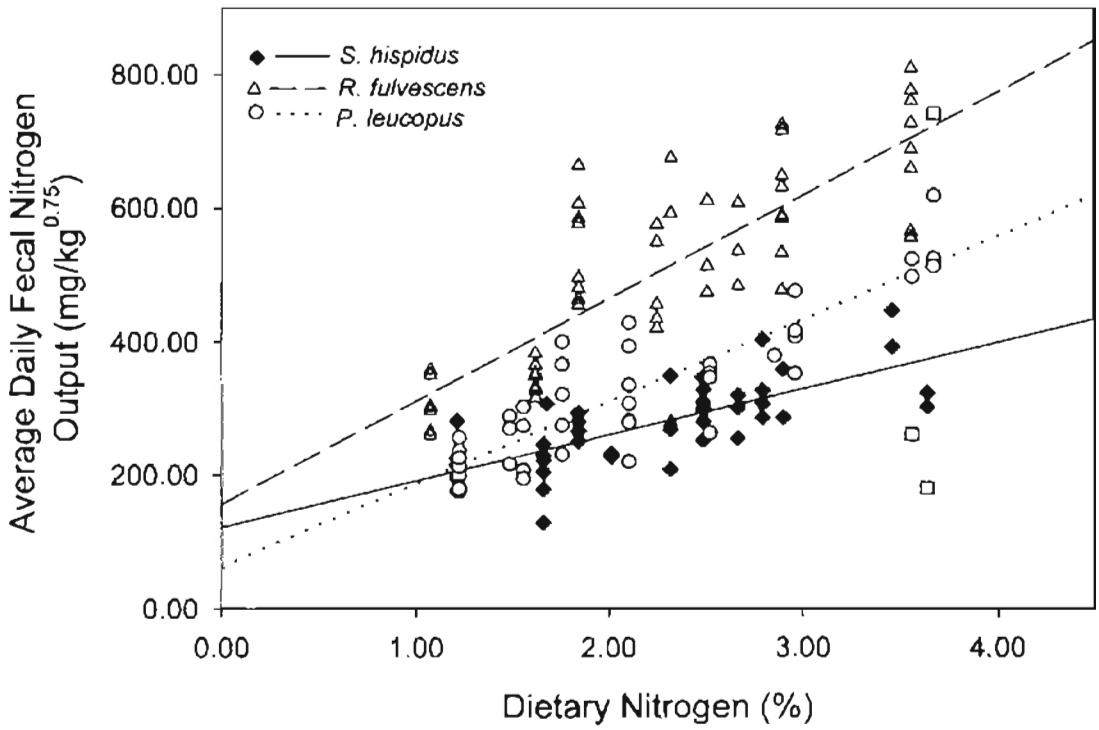
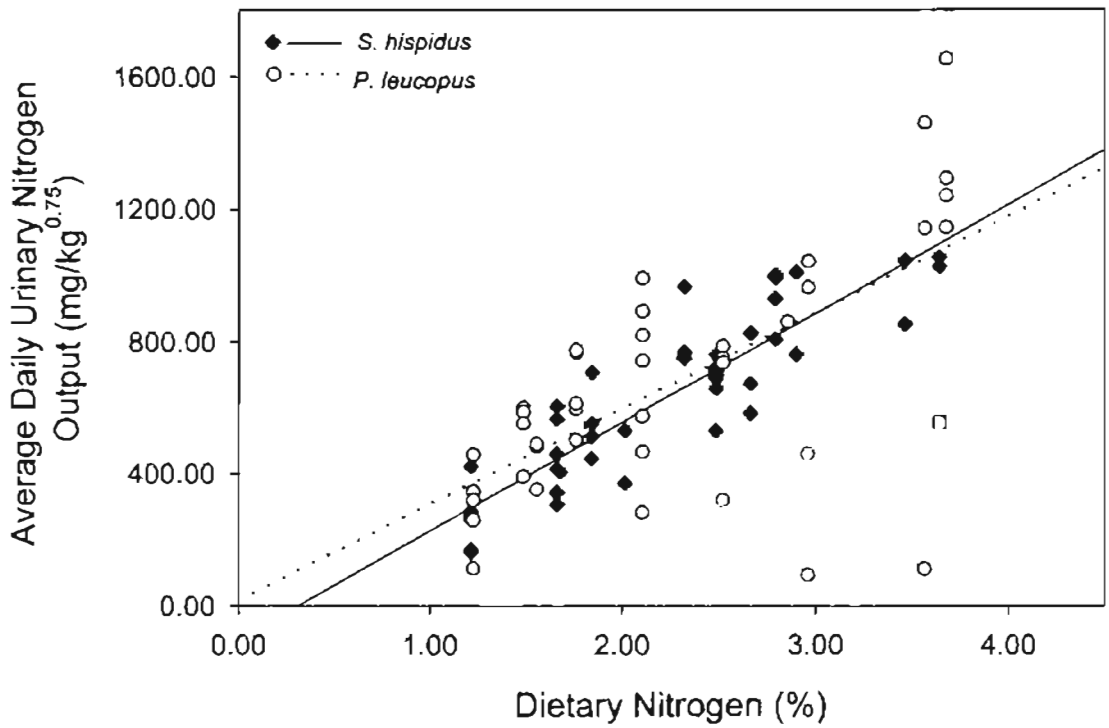

S. hispidus
R. fulvescens
P. leucopus
Average Daily Fecal Nitrogen Output (mg/kg^0.75)
800.00
600.00
400.00
200.00
0.00
0.00
1.00
2.00
3.00
4.00
Dietary Nitrogen (%)

Fig. 8.—Comparison of estimated regression lines between *S. hispidus* (n = 42, Y = -101.87 + 329.32X, $P < 0.001$, $r^2 = 0.79$) and *P. leucopus* (n = 44, Y = 15.78 + 290.97X, $P < 0.001$, $r^2 = 0.44$) for the relationship between urinary nitrogen excretion ($mg/kg^{0.75}/day$) and nitrogen intake (%). The open square represents an extreme value for *S. hispidus* that was excluded from the regression analysis.


S. hispidus
P. leucopus
Average Daily Urinary Nitrogen Output (mg/kg^0.75)
1600.00
1200.00
800.00
400.00
0.00
0.00
1.00
2.00
3.00
4.00
Dietary Nitrogen (%)

The relationships between DMD and dietary nitrogen and between AND and dietary nitrogen differed among all 3 species in my study ($n = 139$, $P \leq 0.005$ for DMD, $P \leq 0.008$ for AND; Figs. 9 and 10). For DMD, intercepts differed little, but *R. fulvescens* had the steepest slope, followed by *P. leucopus* and *S. hispidus*. For AND, slopes were similar, but *R. fulvescens* had the smallest intercept, followed by *P. leucopus* and *S. hispidus*. ANOVA (species by sex) also indicated significant species differences in DMD and AND ($n = 139$, $P < 0.001$; Table 6). A SNK test of these variables showed that the apparent digestive efficiencies of all species differed from each other, with the highest efficiency in *S. hispidus* and the lowest in *R. fulvescens*. Pairwise *Z*-tests of TND showed that *S. hispidus* had higher digestive efficiencies than *R. fulvescens* or *P. leucopus* ($Z = 2.56$, $P = 0.01$ and $Z = 2.33$, $P = 0.02$, respectively; Table 6), but there was no difference between *R. fulvescens* and *P. leucopus* ($Z = 0.50$, $P = 0.62$; Table 6).

Reproductive Requirements

General

Captive-born and wild-caught cotton rats had similar litter sizes ($t_{28} = 0.99$, $P = 0.33$; Table 8); however, captive-born adult females were larger ($t_{13.7} = 2.35$, $P = 0.03$; Table 8) 1 day after parturition than wild-caught females. There was no association ($n = 30$, $r = 0.22$, $P = 0.25$) between the number of days each female was fed experimental rations before parturition (Table 8) and dietary nitrogen level. I also did not find any differences in the length of time wild-caught and captive-born females were fed experimental rations ($t_{13.3} = 1.61$, $P = 0.13$). There were no differences relative to origin for the body mass of harvest mice 2 days after parturition ($t_{8.77} = 1.30$, $P = 0.23$; Table 8) or for average litter size ($t_{28} = -0.88$, $P = 0.39$; Table 8). There was no correlation ($n =$

Fig. 9.—Comparison of estimated regression lines between *S. hispidus* (n = 42, Y = 74.58 + -2.49X, $P < 0.001$, $r^2 = 0.44$), *R. fulvescens* (n = 53, Y = 73.90 – 5.64 X, $P < 0.001$, $r^2 = 0.43$), and *P. leucopus* (n = 44, Y = 73.65 – 3.21X, $P < 0.001$, $r^2 = 0.60$) for the relationship between dry matter digestibility (%) and dietary nitrogen intake (%).

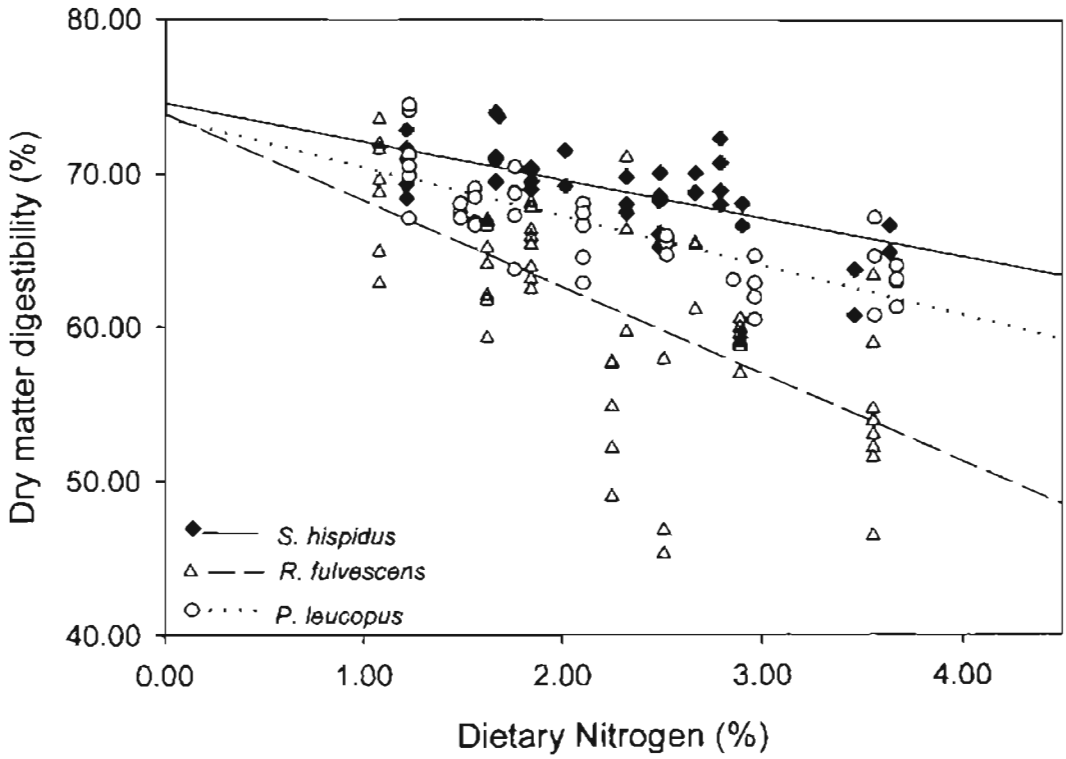

80.00
70.00
60.00
50.00
40.00
Dry matter digestibility (%)
0.00
1.00
2.00
3.00
4.00
Dietary Nitrogen (%)
S. hispidus
R. fulvescens
P. leucopus

Fig. 10.—Comparison of estimated regression lines between *S. hispidus* ($n = 42$, Y = 63.39 + 4.37X, $P < 0.001$, $r^2 = 0.49$), *R. fulvescens* ($n = 53$, Y = 56.42 + 4.20X, $P < 0.001$, $r^2 = 0.35$), and *P. leucopus* ($n = 44$, Y = 60.33 + 3.52X, $P < 0.001$, $r^2 = 0.42$) for the relationship between apparent nitrogen digestibility (%) and dietary nitrogen intake (%).

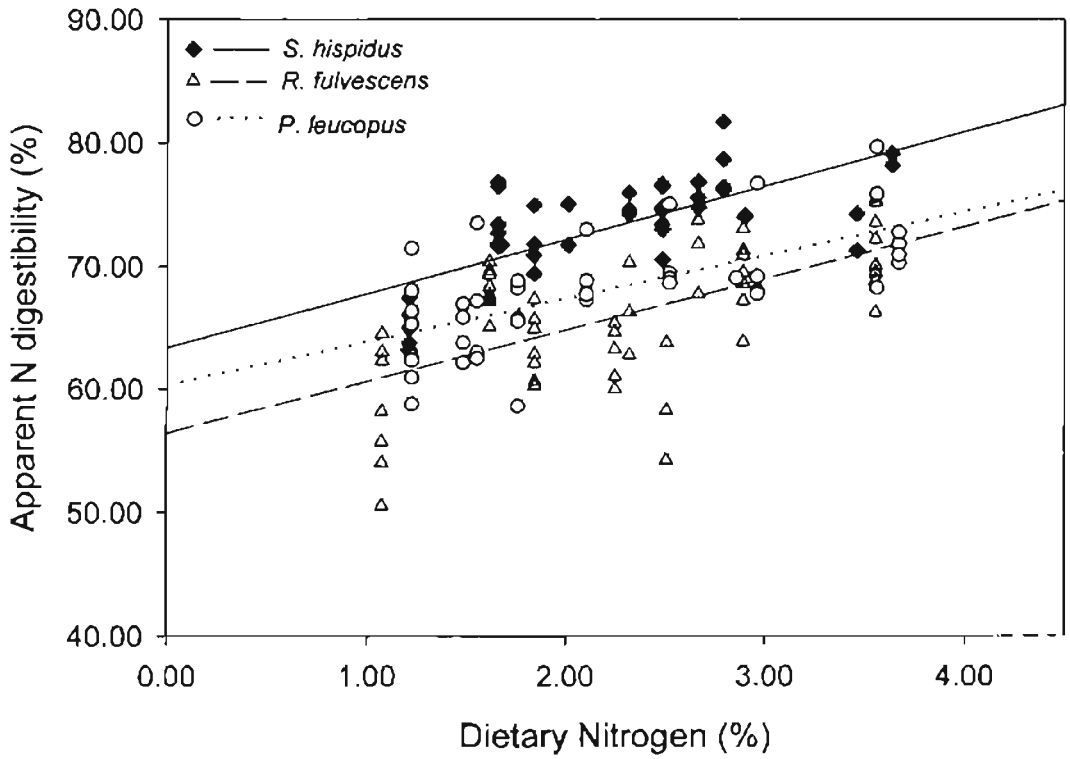

90.00
80.00
70.00
60.00
50.00
40.00
Apparent N digestibility (%)
S. hispidus
R. fulvescens
P. leucopus
0.00
1.00
2.00
3.00
4.00
Dietary Nitrogen (%)

Table 8.—Comparison of data from reproductive feeding trials for *S. hispidus* ($n = 30$) and *R. fulvescens* ($n = 28$)

Variable	Species			
	S. hispidus		*R. fulvescens*	
	$\overline{X}$	*SE*	$\overline{X}$	*SE*
Days on diet before parturition	9.4	0.6	7.0	0.4
Mass of females at parturition (g)	170.17	5.94	15.66	0.24
Litter size	5.47	0.27	3.80	0.21
DMI at given points during lactation ($g/kg^{0.75}$/day):				
Day 5	74.70	2.17	120.67	8.84
Day 10	108.12	3.95	130.70	7.68
Day 15	163.89	10.12	157.89	5.44
Day 20	-----	-----	190.32	13.14
Peak[a]	126.44	4.98	152.49	6.07

[a]Peak lactation = 12 days postpartum for *S. hispidus* and 14 days postpartum for *R. fulvescens*.

30, $r = -0.30$, $P = 0.11$) between the number of days animals were fed experimental rations before parturition (Table 8) and dietary nitrogen, and there were no differences in the amount of time wild-caught and captive-born animals underwent treatment protocols ($t_{28} = -0.15$, $P = 0.88$).

Because animals in the reproductive feeding trial tended to chew their rations into small pieces or powder, I could not accurately measure DMI for all individuals. However, for *S. hispidus* with reliable DMI measurements, I found no relationship between DMI ($g/kg^{0.75}$/day) and nitrogen intake (%) at day 5, 10, or 15 of lactation (day 5: $n = 11$, $P = 0.12$, $r^2 = 0.24$; day 10: $n = 16$, $P = 0.38$, $r^2 = 0.06$; day 15: $n = 11$, $P = 0.13$, $r^2 = 0.24$). I also failed to find a relationship between feed intake and nitrogen intake at any point in lactation for harvest mice (day 5: $n = 7$, $P = 0.48$, $r^2 = 0.11$; day 10: $n = 10$, $P = 0.93$, $r^2 = 0.001$; day 15: $n = 7$, $P = 0.55$, $r^2 = 0.08$; day 20: $n = 7$, $P = 0.91$, $r^2 = 0.003$). Average DMI increased throughout the lactation period for both species (Table 8). Because of the limited amount of data available on DMI, I was unable to test differences between captive-born and wild-caught individuals. A *t*-test indicated that treatments were assigned similarly to captive-born and wild-caught females in both species (*S. hispidus*: $t_{28} = 0.96$, $P = 0.35$; *R. fulvescens*: $t_{28} = -0.59$, $P = 0.56$).

Breakpoint Analysis

Analysis of reproductive data for *S. hispidus* found that neither maternal body mass ($n = 30$, $P = 0.10$, $r^2 = 0.09$) nor litter size ($P = 0.68$, $r^2 < 0.01$) were significant covariates for the relationship between growth rate of individual offspring (g/day) and maternal nitrogen intake (%) at day 12 of lactation. The same analysis of potential

covariates for the relationship between growth rate of total litters (g/day) and maternal nitrogen intake (%) found significant results for both maternal body mass ($P = 0.03$, $r^2 = 0.15$) and litter size ($P < 0.001$, $r^2 = 0.53$). Data on litter growth rates therefore were serially adjusted (litter size followed by maternal mass) for both variables before breakpoint analysis.

Maximum individual growth rates in cotton rats at day 12 of lactation were achieved at 2.31 ± 0.16 % maternal nitrogen intake ($P < 0.001$; Fig. 11; Table 9, Equation 19). Maximal gain in litter mass at day 12 of lactation occurred at a maternal nitrogen intake of 2.62 ± 0.27 % ($P < 0.001$; Fig. 12; Table 9, Equation 20). Using available data on average DMI at 12 days after parturition (126.44 ± 4.98 g DMI/$kg^{0.75}$/day), I estimated that cotton rats would require 2.92 g N/$kg^{0.75}$/day to maximize growth rates of individual offspring and 3.31 g N/$kg^{0.75}$/day to maximize gain in litter mass.

For *R. fulvescens*, maternal body mass ($n = 28$, $P = 0.04$, $r^2 = 0.15$) and litter size ($P = 0.002$, $r^2 = 0.32$) were significant covariates in the relationship between growth rate of individual offspring (g/day) and maternal nitrogen intake (%) at day 14 of lactation. Data were subsequently adjusted for both covariates (first for litter size, then for maternal mass). Litter size ($P = 0.005$, $r^2 = 0.27$), but not maternal body mass ($P = 0.32$, $r^2 = 0.04$), was a covariate in the relationship between litter growth rate (g/day) and % maternal nitrogen intake. Therefore, litter growth rate data were adjusted only for the effect of litter size.

Table 9.—Equations for predicting reproductive performance of *S. hispidus* and *R. fulvescens* under variable % dietary nitrogen.

Equation #	Species	Equation	Parameters of regression model		
			n	*P*	r^2
19	*S. hispidus*	g change in day 12 individual mass/day = -0.01 + 0.83 * (% maternal N intake) for X < 2.31, Y = 1.90 for X > 2.31	30	< 0.001	0.68
20	*S. hispidus*	g change in day 12 litter mass/day = 2.48 + 3.07 * (% maternal N intake) for X < 2.62, Y = 10.51 for X > 2.62	30	< 0.001	0.55
21	*R. fulvescens*	g change in day 14 individual mass/day = -0.80 + 0.81 * (% maternal N intake) for X < 1.29, Y = 0.24 for X > 1.29	28	0.005	0.35
22	*R. fulvescens*	g change in day 14 litter mass/day = -0.71 + 0.94 * (% maternal N intake) for X < 1.68, Y = 0.88 for X > 1.68	28	0.001	0.42
23	*S. hispidus*	number of offspring = 3.94 + 0.66 * (% maternal N intake)	30	0.05	0.13
24	*S. hispidus*	g litter mass at birth = 21.32 + 8.15 * (% maternal N intake)	30	0.001	0.33
25	*S. hispidus*	g weaned litter mass = 56.69 + 52.95 * (% maternal N intake)	30	< 0.001	0.51
26	*S. hispidus*	g individual mass at birth = 6.12 + 0.54 * (% maternal N intake)	30	0.004	0.25
27	*S. hispidus*	g individual weaning mass = 19.79 + 5.60 * (% maternal N intake)	30	< 0.001	0.44
28	*R. fulvescens*	g individual mass at birth = 1.23 + 0.18 * (% maternal N intake)	30	< 0.001	0.35
29	*R. fulvescens*	g individual weaning mass = 4.46 + 1.03 * (% maternal N intake)	28	0.02	0.21

Table 9 (concluded).

Equation #	Species	Equation	Parameters of regression model		
			n	*P*	r^2
30	*R. fulvescens*	log (1/offspring survival rate) = 1.87 – 14.63 * (% maternal N intake)	30	< 0.001	0.84
31	*S. hispidus*	log (g maternal mass lost during lactation) = 1.48 – 0.15 * [log (% maternal N intake)]	30	0.03	0.17
32	*R. fulvescens*	log (g maternal mass lost during lactation) = 1.08 + 0.62 * [log (% maternal N intake)]	28	0.01	0.22

Fig. 11.—Breakpoint analysis of growth rates (g/day) of average 12-day-old *S. hispidus* ($n = 30$; $Y = -0.01 + 0.83X$ for $X < 2.31$, $Y = 1.90$ for $X > 2.31$; $P < 0.001$; $r^2 = 0.68$).

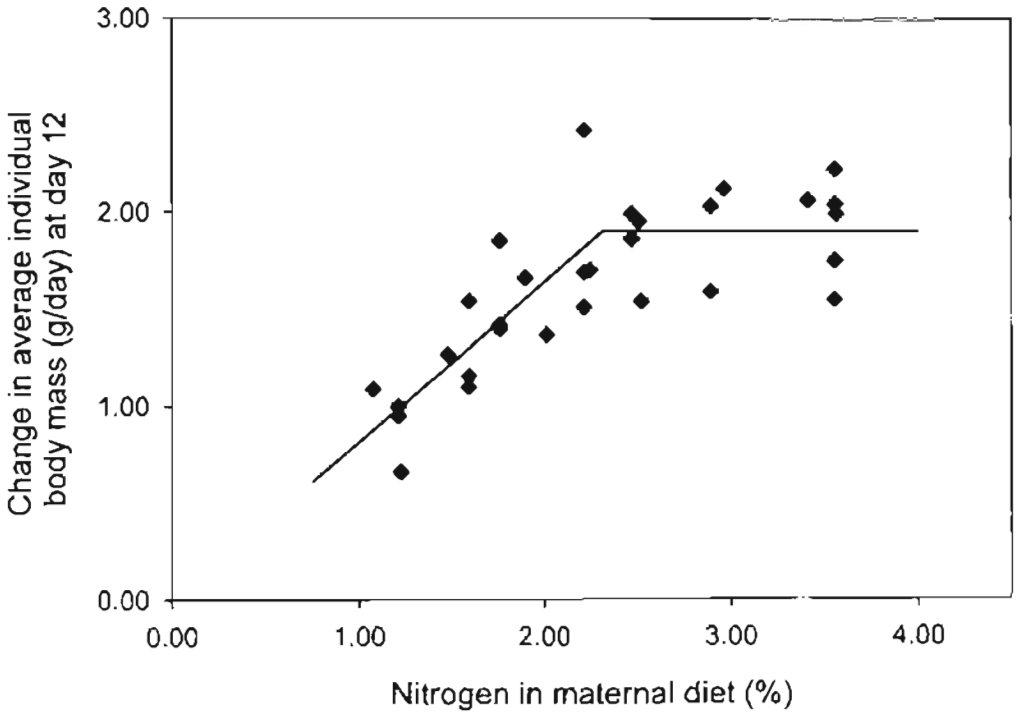
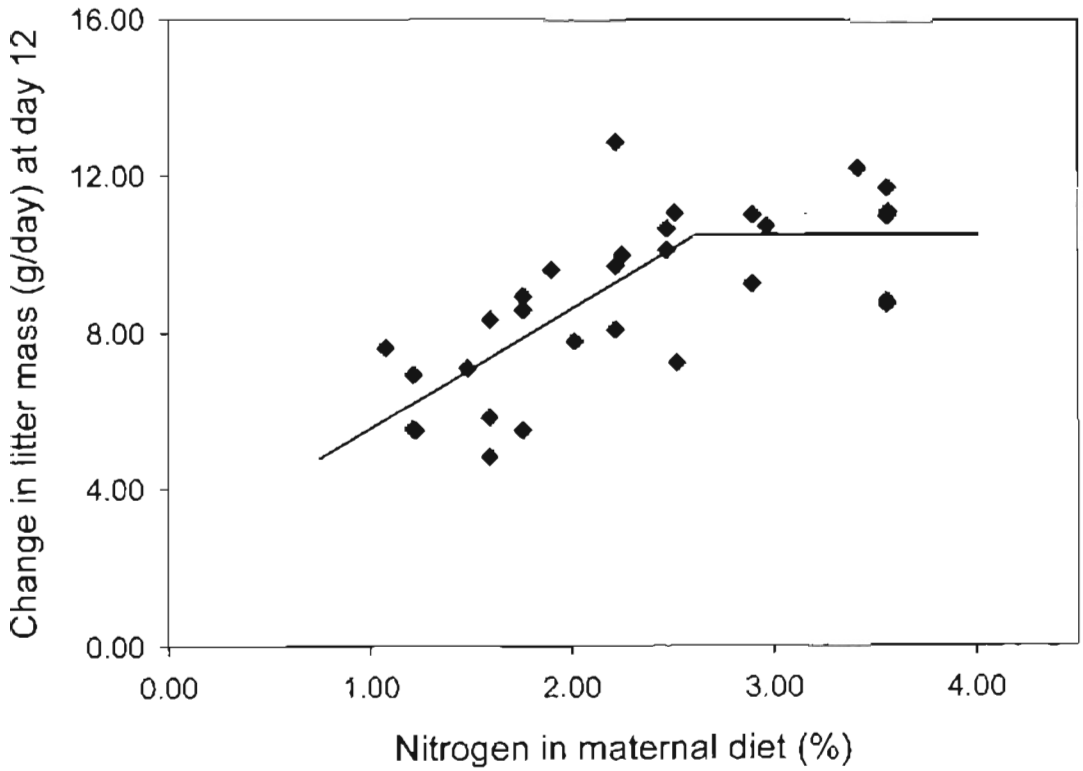

3.00
2.00
1.00
0.00
0.00
1.00
2.00
3.00
4.00
Change in average individual body mass (g/day) at day 12
Nitrogen in maternal diet (%)

Fig. 12.—Breakpoint analysis of litter growth rates (g/day) of 12-day-old *S. hispidus* (n = 30; $Y = 2.48 + 3.07X$ for $X < 2.62$, $Y = 10.51$ for $X > 2.62$; $P < 0.001$, $r^2 = 0.55$).


16.00
12.00
8.00
4.00
0.00
Change in litter mass (g/day) at day 12
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Breakpoint analysis of growth rates of 14-day-old harvest mice showed a requirement of 1.29 ± 0.10 % maternal nitrogen intake ($P = 0.005$; Fig. 13; Table 9, Equation 21). Similar analysis of litter growth rates indicated that maximum growth occurred at a maternal nitrogen intake of 1.68 ± 0.10 % ($P = 0.001$; Fig. 14; Table 9, Equation 22). Harvest mice at 14 days after parturition consumed an estimated 152.49 ± 6.07 g DMI/kg$^{0.75}$/day at peak lactation, thus requiring 1.97 g N/kg$^{0.75}$/day for maximum individual growth rates and 2.56 g N/kg$^{0.75}$/day for maximum litter growth rates.

Reproductive and Survival Responses to Maternal Nitrogen Intake

Litter size was weakly related to maternal nitrogen intake (%) for *S. hispidus* ($P = 0.05$; Table 9, Equation 23). However, the relationship between litter mass (g) and maternal nitrogen intake (%) for cotton rats was significant at birth ($P = 0.001$; Table 9, Equation 24) and weaning ($P < 0.001$; Table 9, Equation 25). Average mass (g) of individual offspring was positively related to maternal nitrogen intake (%) at birth ($P = 0.004$; Table 9, Equation 26) and weaning ($P < 0.001$; Table 9, Equation 27). Analyses using indicator variables showed that the response of all reproductive parameters to maternal nitrogen intake did not vary by origin ($P \geq 0.15$).

Similar analyses for *R. fulvescens* did not show a significant relationship between litter size and % maternal nitrogen intake ($n = 30$, $P = 0.24$, $r^2 = 0.05$). Likewise, the litter mass (g) of harvest mice was not affected by % maternal N intake at birth ($n = 30$, $P = 0.30$, $r^2 = 0.04$) or at weaning ($n = 28$, $P = 0.10$, $r^2 = 0.10$). Regression of average individual body mass (g) on maternal nitrogen intake (%) indicated a significant relationship at birth ($P = 0.001$; Table 9, Equation 28) and weaning ($P = 0.02$; Table 9,

Fig. 13.—Breakpoint analysis of growth rates (g/day) of average 14-day-old *R. fulvescens* (n = 28; Y = -0.80 + 0.81X for X < 1.29, Y = 0.24 for X > 1.29; P = 0.005, r^2 = 0.35).

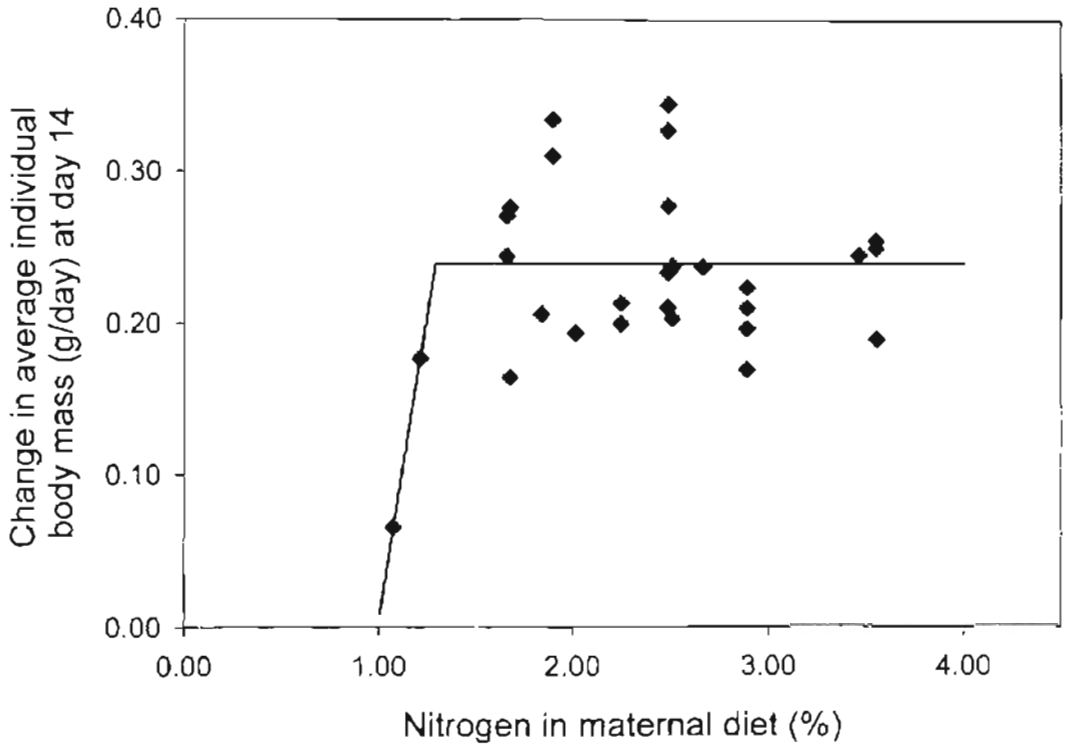
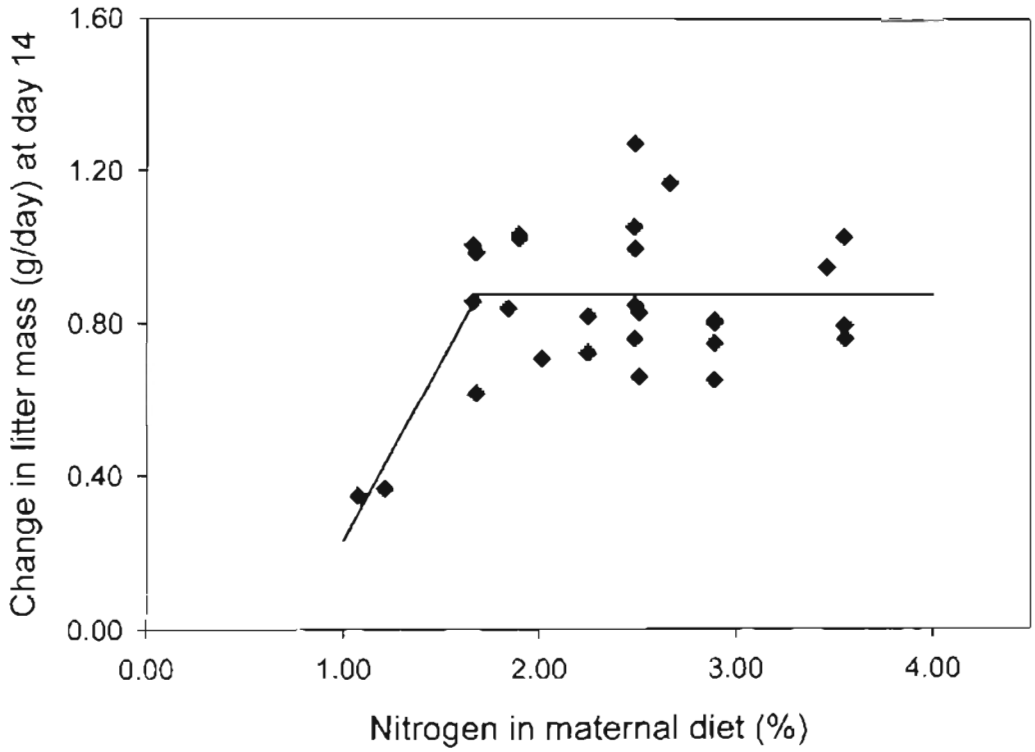

0.40
0.30
0.20
0.10
0.00
Change in average individual body mass (g/day) at day 14
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Fig. 14.—Breakpoint analysis of litter growth rates (g/day) of 14-day-old *R. fulvescens* (n = 28; Y = -0.71 + 0.94X for X < 1.68; Y = 0.88 for X > 1.68; $P = 0.001$, $r^2 = 0.42$).


Change in litter mass (g/day) at day 14
1.60
1.20
0.80
0.40
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Equation 29). Tests of differences between captive-born and wild-caught females showed no difference for any of these variables ($P \geq 0.17$).

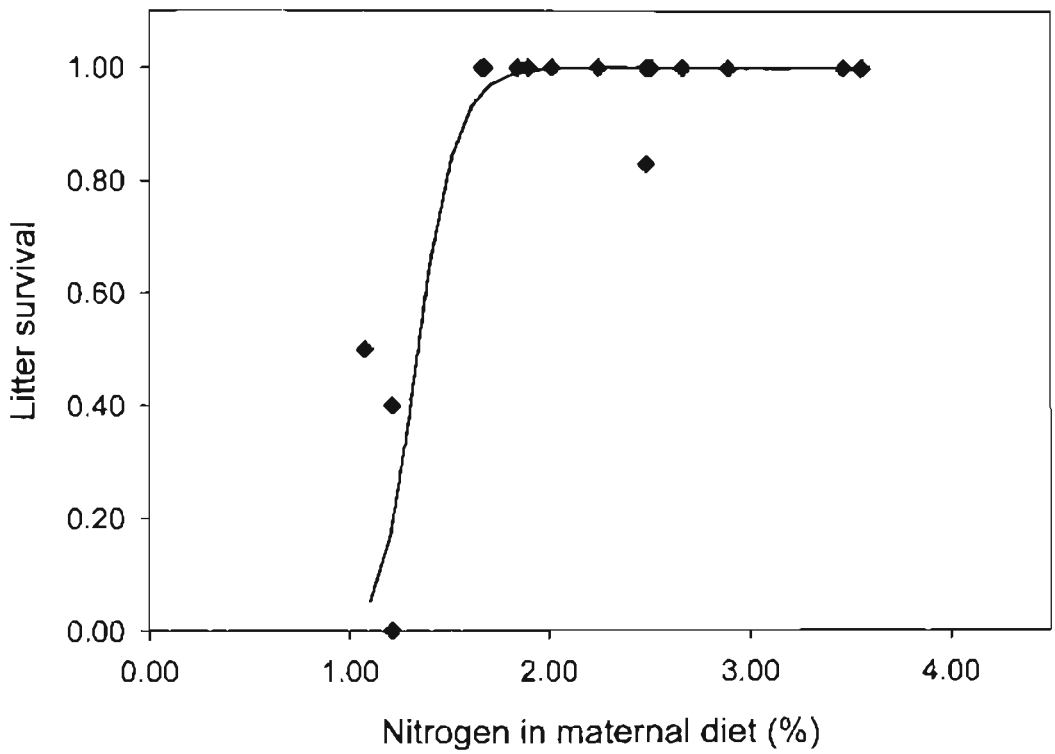
Survival rates of offspring under varying levels of maternal nitrogen intake differed by species. All 164 cotton rat offspring survived until weaning and beyond; no deaths of juvenile cotton rat occurred during the entire study. However, at lower levels of nitrogen intake, some neonatal harvest mice did not survive until weaning or beyond 1 week postweaning. Pups that died before weaning were all consumed by their mothers, but I am not certain whether they were killed by their mother or died from natural causes. Sixteen of 114 harvest mouse pups died before reaching 28 days of age (only 1 of 98 mice that survived until 1 week postweaning died during the 6-week postweaning period). According to a logistic equation predicting within-litter survival rates for *R. fulvescens* ($P < 0.001$; Fig. 15; Table 9, Equation 30), maternal nitrogen intake necessary for a survival rate of 0.5 was 1.34 ± 0.08 %.

Interspecific Comparisons

Harvest mice had greater metabolic body mass-specific DMI at the beginning of lactation (day 5; $t_{11.2} = 5.05$, $P < 0.001$) and at peak lactation ($t_{21} = 3.04$, $P = 0.006$; Table 8) than cotton rats. At the end of the lactation period (day 15 for *S. hispidus* and day 20 for *R. fulvescens*), there was no species difference in DMI ($t_{18} = 1.61$, $P = 0.12$; Table 8). Cotton rats produced larger litters ($t_{58} = -4.91$, $P < 0.001$; Table 8) than harvest mice.

Breakpoint analyses showed that *S. hispidus* required more dietary nitrogen than *R. fulvescens* to achieve maximum growth rate of individual pups ($Z = 5.41$, $P < 0.001$) and whole litters ($Z = 3.29$, $P = 0.001$). Analyses of the responses of reproductive

Fig. 15.—Within-litter survival rates over varying levels of maternal nitrogen intake (%). Survival was 1.0 for all *S. hispidus* offspring. The estimated regression equation for *R. fulvescens* is $\log (1/Y) = 1.87 - 14.63X$ ($n = 30$, $P < 0.001$, $r^2 = 0.84$).


1.00
0.80
0.60
0.40
0.20
0.00
Litter survival
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

parameters to % maternal nitrogen intake indicated species differences for litter size (n = 60, $P < 0.001$; Fig. 16), litter mass at birth (n = 60, $P < 0.001$; Fig. 17), litter mass at weaning (n = 58, $P < 0.001$; Fig. 18), average mass of individuals at birth (n = 60, $P < 0.001$; Fig. 19), and individual mass at weaning (n = 58, $P < 0.001$; Fig. 20). In all cases but one (that of individual pup mass at birth), cotton rats exhibited stronger reproductive responses to % maternal nitrogen than harvest mice. Maternal mass lost (%) as a function of % nitrogen intake varied between species, with *S. hispidus* losing proportionally more mass ($P < 0.001$) than *R. fulvescens*. Cotton rats also increased mass loss with decreasing dietary nitrogen ($P = 0.03$; Fig. 21; Table 9, Equation 31), whereas the opposite relationship was seen in harvest mice ($P = 0.01$; Fig. 21; Table 9, Equation 32).

Three litters of *Peromyscus* were born during the reproductive trials to females consuming 1.22 %, 2.10 %, and 3.56 % nitrogen. Although I was unable to conduct analyses on this species, I collected descriptive data on the growth and development of those litters. The lowest-nitrogen litter had ≥ 4 pups, 3 of which were alive on the day of their birth (I observed the remains of 1 pup in addition to the 3 live young). However, by 2 days after parturition, all offspring had died of unknown causes and been cannibalized by their mother. The female receiving 2.10 % dietary nitrogen gave birth to 4 pups with an average birth mass of 2.83 g that grew at a rate of 0.5 g/day at peak lactation (at which time feeding trials were discontinued). The female consuming 3.56% nitrogen had a litter of 2 pups with an average birth mass of 3.00 g. That litter grew at a rate of 0.6 g/day at peak lactation and had an average weaning mass of 12.4 g. Young of both surviving litters reached key developmental stages (e.g., growing fur, opening their eyes,

Fig. 16.—Relationships between litter size and % maternal nitrogen intake in *S. hispidus* ($n = 30$, $Y = 3.94 + 0.66X$, $P = 0.05$, $r^2 = 0.13$) and *R. fulvescens* ($n = 30$, $Y = 4.60 - 0.35X$, $P = 0.24$, $r^2 = 0.05$).

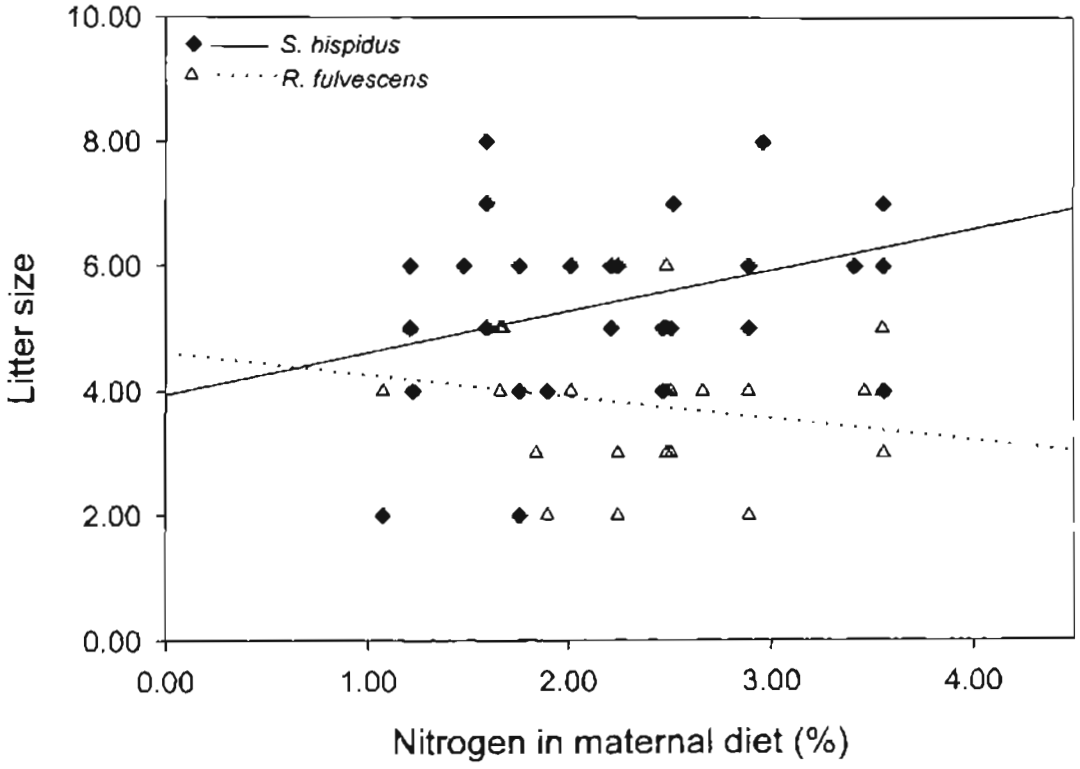

10.00
8.00
6.00
4.00
2.00
0.00
Litter size
S. hispidus
R. fulvescens
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Fig. 17.—Relationships between litter mass at birth (g) and % maternal nitrogen intake in *S. hispidus* ($n = 30$, $Y = 21.32 + 8.15X$, $P = 0.001$, $r^2 = 0.33$) and *R. fulvescens* ($n = 30$, $Y = 5.80 + 0.49X$, $P = 0.30$, $r^2 = 0.04$).

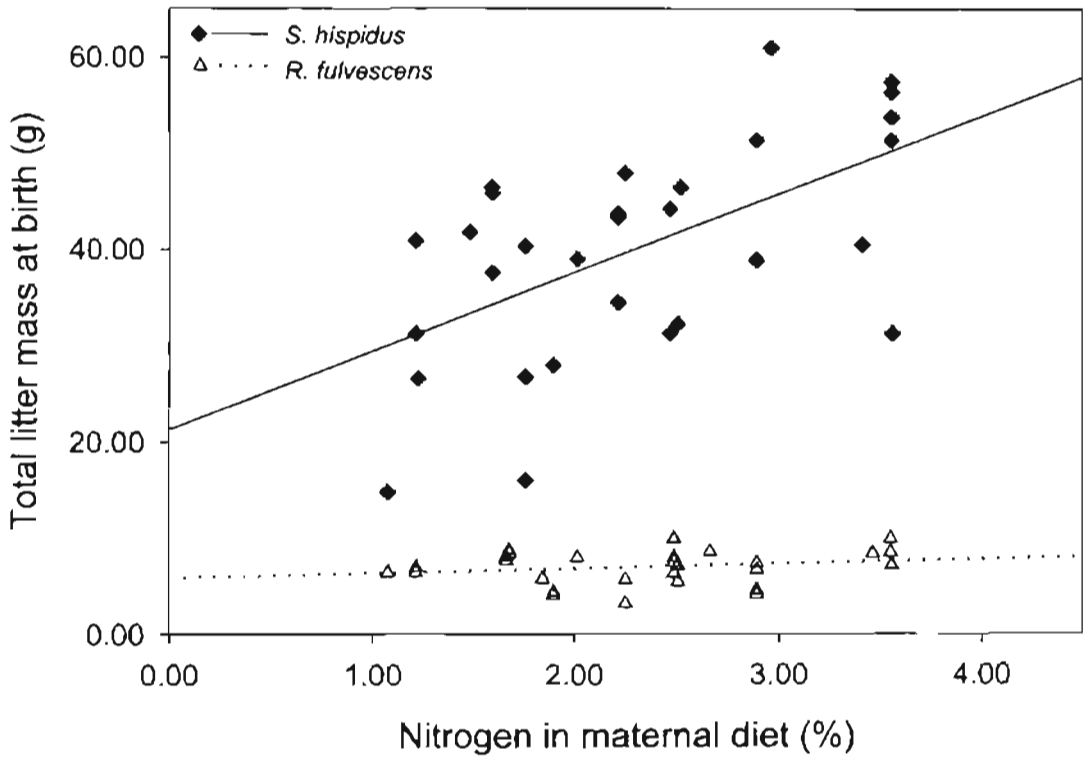

S. hispidus
R. fulvescens
60.00
40.00
20.00
0.00
Total litter mass at birth (g)
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Fig. 18.—Relationship between litter mass at weaning (g) and % maternal nitrogen intake in *S. hispidus* ($n = 30$, $Y = 56.69 + 52.95X$, $P < 0.001$, $r^2 = 0.51$) and *R. fulvescens* ($n = 28$, $Y = 17.16 + 3.20X$, $P = 0.10$, $r^2 = 0.10$).

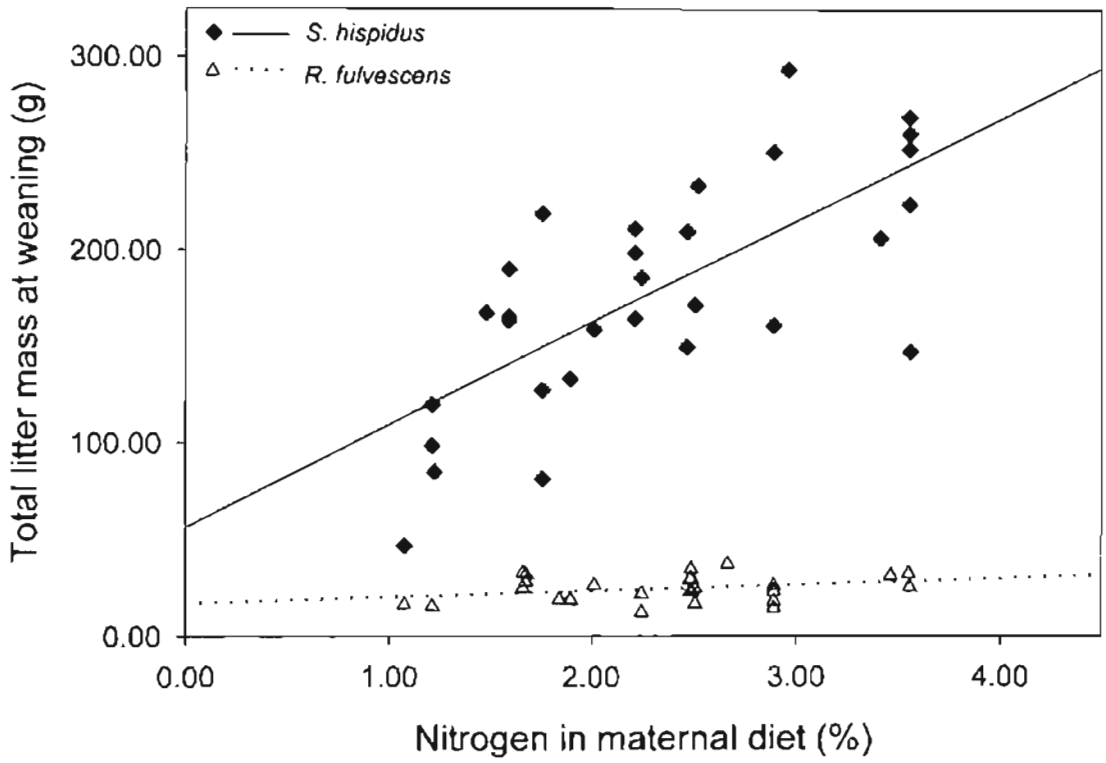

S. hispidus
R. fulvescens
300.00
200.00
100.00
0.00
Total litter mass at weaning (g)
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Fig. 19.—Relationships between individual mass at birth (g) and % maternal nitrogen intake in *S. hispidus* ($n = 30$, $Y = 6.12 + 0.54X$, $P = 0.004$, $r^2 = 0.25$) and *R. fulvescens* ($n = 30$, $Y = 1.23 + 0.18X$, $P < 0.001$, $r^2 = 0.35$).

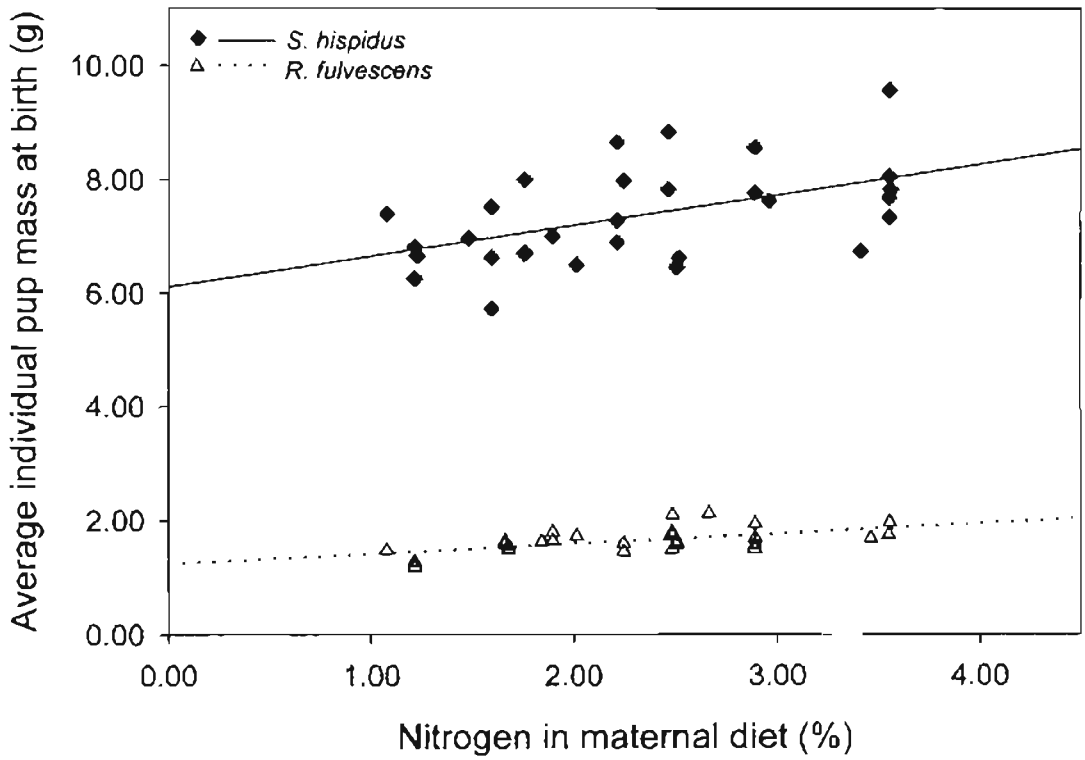

S. hispidus
R. fulvescens
Average individual pup mass at birth (g)
10.00
8.00
6.00
4.00
2.00
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Fig. 20.—Relationships between individual mass at weaning (g) and % maternal nitrogen intake in *S. hispidus* ($n = 30$, $Y = 19.79 + 5.60X$, $P < 0.001$, $r^2 = 0.44$) and *R. fulvescens* ($n = 28$, $Y = 4.46 + 1.03X$, $P = 0.02$, $r^2 = 0.21$).

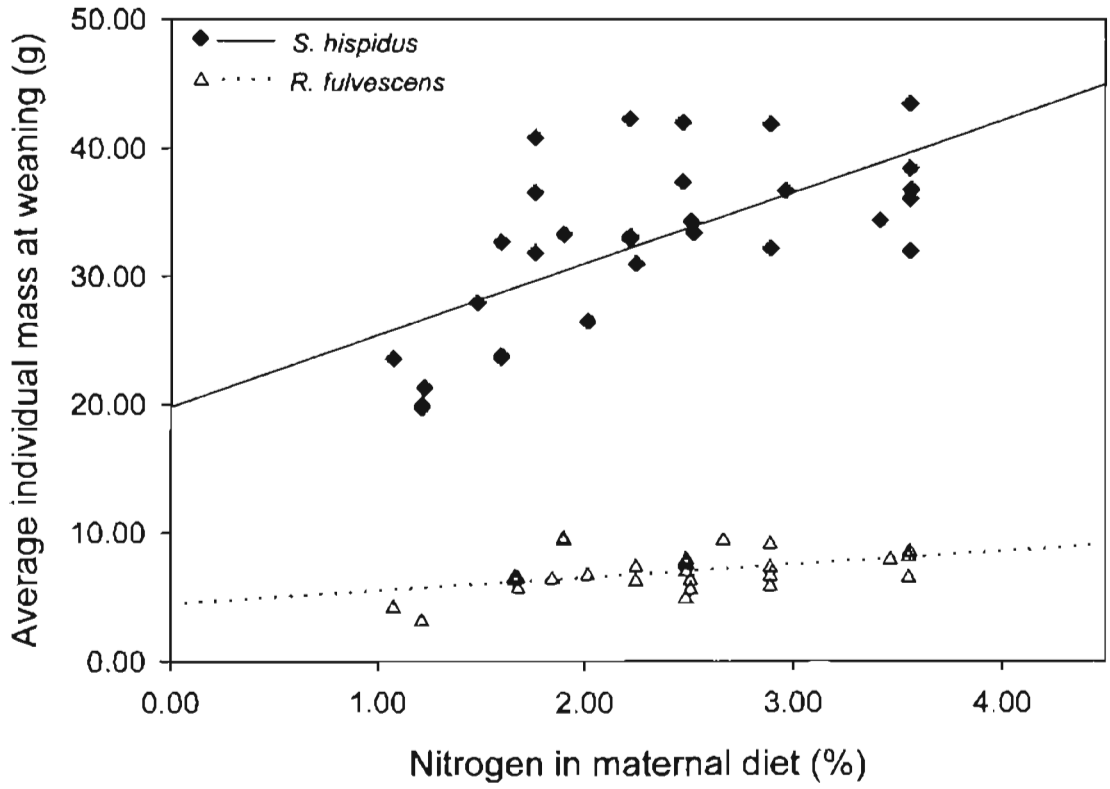
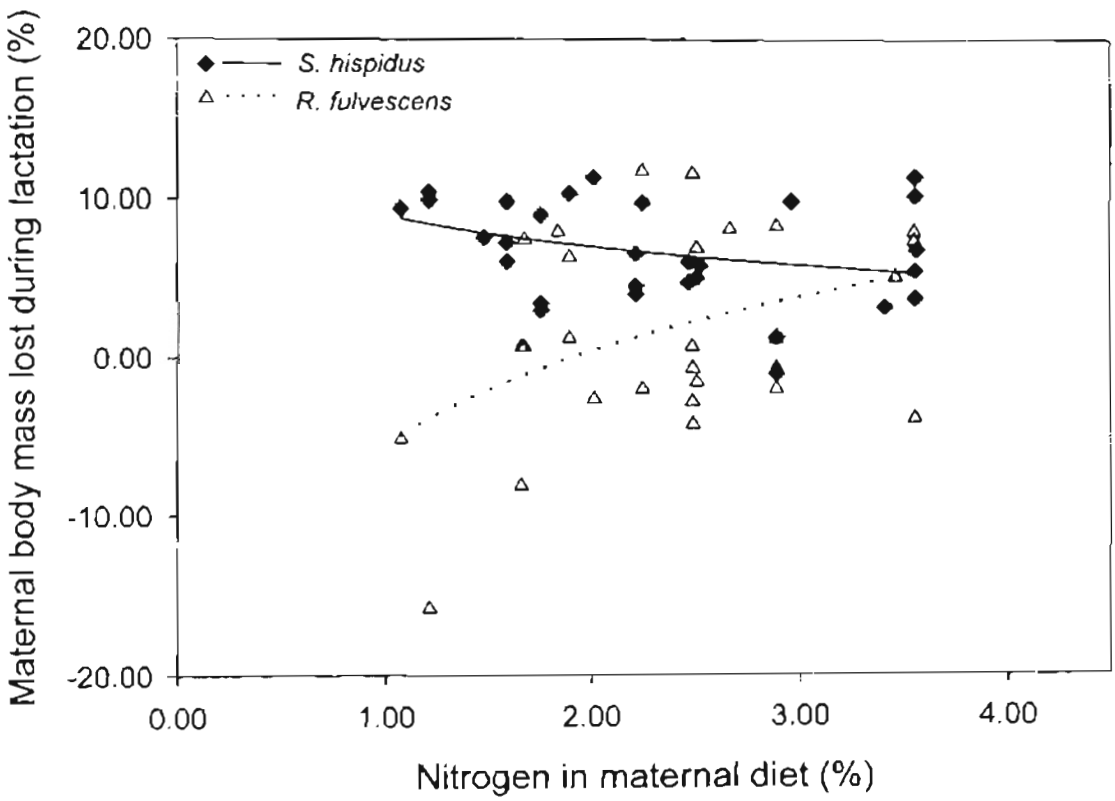

S. hispidus
R. fulvescens
Average individual mass at weaning (g)
50.00
40.00
30.00
20.00
10.00
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Fig. 21.—Relationships between maternal body mass lost during the lactation period (g) and % maternal nitrogen intake in *S. hispidus* ($n = 30$, $\log Y = 1.48 - 0.15(\log X)$, $r^2 = 0.17$) and *R. fulvescens* ($n = 28$, $\log Y = 1.09 + 0.62(\log X)$, $r^2 = 0.22$).


S. hispidus
R. fulvescens
Maternal body mass lost during lactation (%)
20.00
10.00
0.00
-10.00
-20.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

displaying climbing behavior) within one day of each other.

Postweaning Growth

General/Estimation of Requirements

I was unable to accurately quantify DMI for some juveniles undergoing feeding trials because of their tendency to excessively chew experimental rations (as was the case in reproductive trials). Analyses of DMI for both species were performed on a subset of individuals that demonstrated reliable intake rates.

There was a weak negative relationship between DMI ($g/kg^{0.75}$/day) and dietary nitrogen (%) for *S. hispidus* at 1 week postweaning ($n = 47$, $P = 0.04$, $r^2 = 0.09$), but not at week 2 ($n = 29$, $P = 0.62$, $r^2 < 0.01$) or week 3 ($n = 27$, $P = 0.16$, $r^2 = 0.08$). This regression differed by maternal origin (captive-born vs. wild-caught) at 1 week postweaning ($P = 0.008$; young of captive-born cotton rats showed a smaller intercept and a slightly lower slope); however, no other differences in the response of DMI to nitrogen intake were found at any time interval ($P \geq 0.11$). Offspring of wild-caught females showed higher mean DMI at week 1 ($P = 0.001$), but no effect of sex or maternal origin were found at 2 weeks after weaning ($P \geq 0.76$). I found a weak interaction ($P = 0.07$) between these variables at 3 weeks postweaning, with male offspring of wild-caught cotton rats showing higher intake rates than female offspring, but no other effects ($P \geq 0.12$). Calculated separately at 1 week after weaning, young of wild-caught females had an average daily DMI of 89.93 ± 3.32 $g/kg^{0.75}$ and offspring of captive-born females consumed 77.11 ± 1.51 g $DMI/kg^{0.75}$/day. Average DMI for this species decreased from week 1 to week 3 postweaning ($P < 0.03$; Table 10).

R. fulvescens showed no relationships between DMI ($g/kg^{0.75}$/day) and dietary nitrogen (%) at week 1 ($n = 52$, $P = 0.58$, $r^2 < 0.01$), week 2 ($n = 39$, $P = 0.83$, $r^2 < 0.01$), or week 3 ($n = 34$, $P = 0.38$, $r^2 = 0.02$). There also was no influence of sex or maternal origin for any of the 3 regressions ($P \geq 0.20$). I did not find an effect of either of those variables on mean DMI at week 1 or 2 postweaning ($P \geq 0.34$); however, I did find a significant sex effect at week 3 ($P = 0.03$). Average daily DMI at that time was 114.63 ± 4.83 $g/kg^{0.75}$ for male harvest mice and 126.82 ± 5.10 $g/kg^{0.75}$ for females. Mean DMI for *R. fulvescens* showed a slight increase between from week 1 to week 2 postweaning, then decreased at week 3 (Table 10), although none of these differences were significant ($P \leq 0.16$).

Before I carried out breakpoint analyses to estimate growth requirements, I regressed each dependent variable on maternal nitrogen intake to test for the significance of maternal diet as a covariate, with indicator variables to test for sex or maternal origin effects on covariate relationships. The only case in which maternal nitrogen intake was a covariate was for the growth rate of *R. fulvescens* in the first week postweaning; therefore, data were corrected for maternal diet in breakpoint analysis. All other data sets were left uncorrected.

Data on growth rates for *R. fulvescens* at weeks 1 and 2 postweaning did not fit the model for breakpoint analysis (the model failed to converge), and data from week 3 did not yield a significant model ($n = 69$, $P = 0.11$). The breakpoint model also failed to

Table 10.—Comparison of data from postweaning growth trials for *S. hispidus* (n = 101) and *R. fulvescens* (n = 64).

Variable	Species			
	S. hispidus		*R. fulvescens*	
	$\bar{X}$	*SE*	$\bar{X}$	*SE*
Week 1 postweaning				
Dry matter intake (DMI; g/kg$^{0.75}$/day)	82.57	1.88	126.55	4.19
Growth rate (g/day)	2.12	0.05	0.23	0.01
Nitrogen conversion (g gain/g N intake/ kg$^{0.75}$)	1.10	0.06	0.08	0.005
Week 2 postweaning				
DMI (g/kg$^{0.75}$/day)	76.56	2.06	129.99	5.51
Growth rate (g/day)	1.83	0.05	0.20	0.01
Nitrogen conversion (g gain/g N intake/ kg$^{0.75}$)	0.95	0.07	0.07	0.007
Week 3 postweaning				
DMI (g/kg$^{0.75}$/day)	69.08	1.51	120.73	3.62
Growth rate (g/day)	1.60	0.08	0.15	0.01
Nitrogen conversion (g gain/g N intake/ kg$^{0.75}$)	0.89	0.08	0.05	0.005
Adult mass (g)[a]	142.38	7.14	13.13	0.31
Time to adult mass (days)[a]	87.57	4.90	47.47	0.90
Maximum growth rate (g/day)[a]	1.93	0.07	0.30	0.01

[a]Estimated from individual growth curves

converge on growth rate data from week 3 for *S. hispidus*. Therefore, I was only able to analyze data on growth rates of *S. hispidus* from the first 2 weeks postweaning with breakpoint methods. There was no linear relationship between % nitrogen intake and growth rate (g/day) for *R. fulvescens* at any time or for *S. hispidus* at week 3 postweaning ($P \geq 0.11$). Average growth rates for both species decreased from 1 week to 3 weeks after weaning (Table 10).

Maximum growth rates of juvenile cotton rats occurred at 1.96 ± 0.15 % dietary nitrogen ($P < 0.001$; Fig. 22; Table 11, Equation 33) at week 1 postweaning and 1.95 ± 0.27 % dietary nitrogen ($P = 0.01$; Fig. 23; Table 11, Equation 34) at 2 weeks postweaning. Using data on average DMI at these 2 points in time, I estimated that cotton rats would need to consume 1.62 $g/kg^{0.75}$/day and 1.49 $g/kg^{0.75}$/day (at week 1 and 2, respectively) to grow maximally.

Maternal/Individual Effects on Growth

Multiple regression analysis showed that the relationship between body mass (g) of *S. hispidus* at week 3 and 6 postweaning and dietary nitrogen (%) and between mass gain during the first 3 weeks after weaning (g) and nitrogen intake differed according to weanling sex (all $P < 0.001$). Therefore, regression models were developed separately for males and females.

Body mass (g) of male cotton rats at 3 weeks postweaning was related to the interaction between % maternal and postweaning nitrogen intake (i.e., the product of the 2 independent variables; $P < 0.001$; Fig. 24A; Table 11, Equation 35; Table 12), whereas

Table 11.—Equations for predicting postweaning growth of *S. hispidus* and *R. fulvescens* under variable % maternal and postweaning dietary nitrogen.

Equation #	Species	Equation	Parameters of regression model		
			n	*P*	r^2/R^2
33	*S. hispidus*	g change in juvenile body mass/day during week 1 postweaning = 0.24 + 1.04 * (% postweaning N intake) for X < 1.96; Y = 2.28 for X > 1.96	101	< 0.001	0.28
34	*S. hispidus*	g change in juvenile body mass/day during week 2 postweaning = 0.77 + 0.59 * (% postweaning N intake) for X < 1.95; Y = 1.92 for X > 1.95	100	0.01	0.10
35	*S. hispidus*	body mass of males at 3 weeks postweaning (g) = 64.60 + 1.97 * (% maternal N intake) * (% postweaning N intake)	44	< 0.001	0.26
36	*S. hispidus*	body mass of females at 3 weeks postweaning (g) = 54.69 + 4.66 * (% maternal N intake)	54	< 0.001	0.20
37	*S. hispidus*	body mass of males at 6 weeks postweaning (g) = 80.95 + 6.25 * (% maternal N intake)	39	0.04	0.11
38	*S. hispidus*	body mass of females at 6 weeks postweaning (g) = 74.21 + 1.25 * (% maternal N intake)^2	44	0.01	0.13
39	*S. hispidus*	g mass gained by males during first 3 weeks postweaning = 32.37 + 4.41 * (% postweaning N intake)	44	0.01	0.17

Table 11 (continued).

Equation #	Species	Equation	Parameters of regression model		
			n	*P*	r^2/R^2
40	*S. hispidus*	g mass gained by females during first 3 weeks postweaning = 15.48 + 14.63 * (% postweaning N intake) – 2.62 * (% postweaning N intake)^2	54	0.06	0.10
41	*S. hispidus*	g adult mass for offspring of wild-caught females = 48.59 + 54.04 * (% maternal N intake) – 8.92 * (% maternal N intake) * (% postweaning N intake)	39	< 0.001	0.42
42	*S. hispidus*	days to adult mass for offspring of wild-caught females = 183.53 – 111.28 * (% postweaning N intake) + 15.86 * (% maternal N intake) + 19.28 * (% postweaning N intake)^2	38	< 0.001	0.37
43	*S. hispidus*	days to adult mass for offspring of captive-born females = 280.88 – 134.51 * (% postweaning N intake) + 22.62 * (% postweaning N intake)^2	39	0.02	0.19
44	*S. hispidus*	g/day gain in mass at maximum growth rate = 2.80 – 0.55 * (% maternal N intake) + 0.08 * (% maternal N intake) * (% postweaning N intake)	79	< 0.001	0.24
45	*S. hispidus*	g daily gain in body mass per g daily nitrogen intake/kg$^{0.75}$ during week 1 postweaning = 2.10 – 0.36 * (% postweaning N intake)	37	< 0.001	0.46

Table 11 (continued).

Equation #	Species	Equation	Parameters of regression model		
			n	*P*	r^2/R^2
46	*S. hispidus*	g daily gain in body mass per g daily nitrogen intake/kg$^{0.75}$ during week 2 postweaning = 1.94 – 0.34 * (% postweaning N intake)	26	0.001	0.36
47	*S. hispidus*	g daily gain in body mass per g daily nitrogen intake/kg$^{0.75}$ during week 3 postweaning = 0.37 + 0.48 * (% maternal N intake) – 0.10 * (% postweaning N intake) * (% maternal N intake)	25	< 0.001	0.47
48	*R. fulvescens*	body mass of males at 3 weeks postweaning (g) = 5.00 + 5.20 * (% maternal N intake) – 0.85 * (% maternal N intake)^2	35	0.003	0.31
49	*R. fulvescens*	body mass of females at 3 weeks postweaning (g) = 11.70 – 0.51 * (% maternal N intake)	34	0.08	0.09
50	*R. fulvescens*	days to adult mass = 45.81 + 2.15 * (% postweaning N intake)^2 + 1.43 * (% maternal N intake)^2 – 3.57 * (% postweaning N intake) * (% maternal N intake)	64	0.04	0.13
51	*R. fulvescens*	g/day gain in mass at maximum growth rate = 0.41 – 0.04 * (% maternal N intake)	64	0.05	0.06
52	*R. fulvescens*	g daily gain in body mass per g daily nitrogen intake/kg$^{0.75}$ during week 1 postweaning = 0.20 – 0.03 * (% postweaning N intake) – 0.01 * (% maternal N intake)	52	< 0.001	0.48

Table 11 (concluded).

Equation #	Species	Equation	Parameters of regression model		
			n	*P*	r^2/R^2
53	*R. fulvescens*	g daily gain in body mass of males per g daily nitrogen intake/$kg^{0.75}$ during week 2 postweaning = 0.14 – 0.01 * (% postweaning N intake)^2	20	0.002	0.43
54	*R. fulvescens*	g daily gain in body mass of females per g daily nitrogen intake/$kg^{0.75}$ during week 2 postweaning = 0.11 – 0.01 * (% postweaning N intake) * (% maternal N intake)	19	0.002	0.43
55	*R. fulvescens*	g daily gain in body mass per g daily nitrogen intake/$kg^{0.75}$ during week 3 postweaning = 0.07 – 0.003 * (% postweaning N intake)^2	34	0.02	0.16

Table 12.—Results of multiple regression analyses of the effects of % maternal and postweaning nitrogen intake on the growth of juvenile *S. hispidus* and *R. fulvescens*. Significant independent variables are indicated (+ or – indicates direction of effect; + = $0.15 > P > 0.05$; + + = $0.05 > P > 0.01$; + + + = $P < 0.01$).

Dependent variable	Potential independent variables				
	% maternal N intake	(% maternal N intake)^2	% postweaning N intake	(% postweaning N intake)^2	(% maternal N intake) x (% postweaning N intake)
S. hispidus					
Body mass at 3 weeks postweaning (g)					
Males					+ + +
Females	+ + +				
Body mass at 6 weeks postweaning (g)					
Males	+ +				
Females		+ +			

Table 12 (continued).

Dependent variable	Potential independent variables				
	% maternal N intake	(% maternal N intake)^2	% postweaning N intake	(% postweaning N intake)^2	(% maternal N intake) x (% postweaning N intake)
S. hispidus					
Mass gained during the first 3 weeks post-weaning (g)					
Males			+ + +		
Females			+	−	
Adult mass (g)					
Offspring of wild-caught females	+ + +				− − −
Time to adult mass (days)					
Offspring of wild-caught females	+ +		− − −	+ +	
Offspring of captive-born females			− −	+	
Maximum growth rate (g/day)	− − −				+ +

Table 12 (continued).

Dependent variable	Potential independent variables				
	% maternal N intake	(% maternal N intake)^2	% postweaning N intake	(% postweaning N intake)^2	(% maternal N intake) x (% postweaning N intake)
S. hispidus					
N conversion during the 1st week postweaning (g gain/g N intake/kg$^{0.75}$)			– – –		
N conversion during the 2nd week postweaning (g gain/g N intake/kg$^{0.75}$)			– – –		
N conversion during the 3rd week postweaning (g gain/g N intake/kg$^{0.75}$)	+ + +				– – –

Table 12 (continued).

Dependent variable	Potential independent variables				
	% maternal N intake	(% maternal N intake)^2	% postweaning N intake	(% postweaning N intake)^2	(% maternal N intake) x (% postweaning N intake)
R. fulvescens					
Body mass at 3 weeks postweaning (g)					
Males	+ +	– –			
Females	–				
Body mass at 6 weeks postweaning (g)					
Mass gained during the first 3 weeks post-weaning (g)					
Adult mass (g)					
Time to adult mass (days)		+		+ +	– –
Maximum growth rate (g/day)	–				

Table 12 (concluded).

Dependent variable	Potential independent variables				
	% maternal N intake	(% maternal N intake)^2	% postweaning N intake	(% postweaning N intake)^2	(% maternal N intake) x (% postweaning N intake)
R. fulvescens					
N conversion during the 1st week postweaning (g gain/g N intake/kg$^{0.75}$)	–		– – –		
N conversion during the 2nd week postweaning (g gain/g N intake/kg$^{0.75}$)					
Males				– – –	
Females					– – –
N conversion during the 3rd week postweaning (g gain/g N intake/kg$^{0.75}$)				– –	

Fig. 22.—Breakpoint analysis of growth rates (g/day) of juvenile *S. hispidus* during the first week postweaning (n = 101; Y = 0.24 + 1.04X for X < 1.96; Y = 2.28 for X > 1.96; $P < 0.001$; $r^2 = 0.28$).

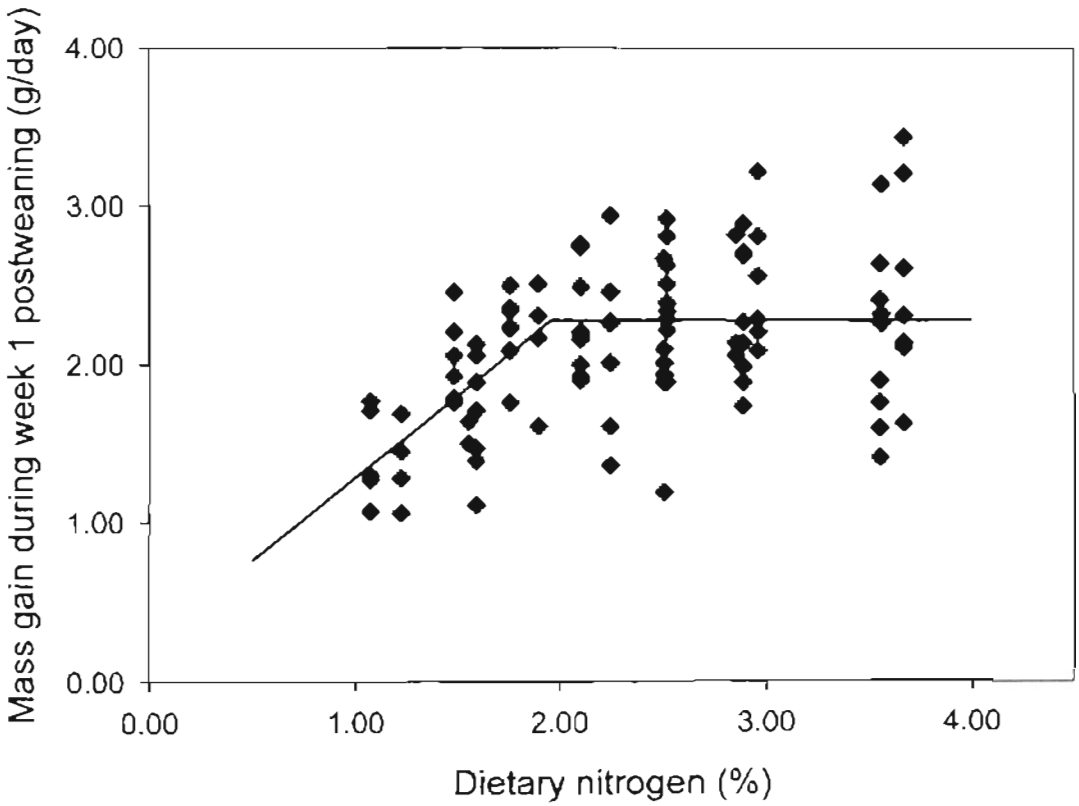

Mass gain during week 1 postweaning (g/day)
4.00
3.00
2.00
1.00
0.00
0.00
1.00
2.00
3.00
4.00
Dietary nitrogen (%)

Fig. 23.—Breakpoint analysis of growth rates of juvenile *S. hispidus* during the second week postweaning (n = 100; Y = 0.77 + 0.59X for X < 1.95; Y = 1.92 for X > 1.95; P = 0.01; r^2 = 0.10).

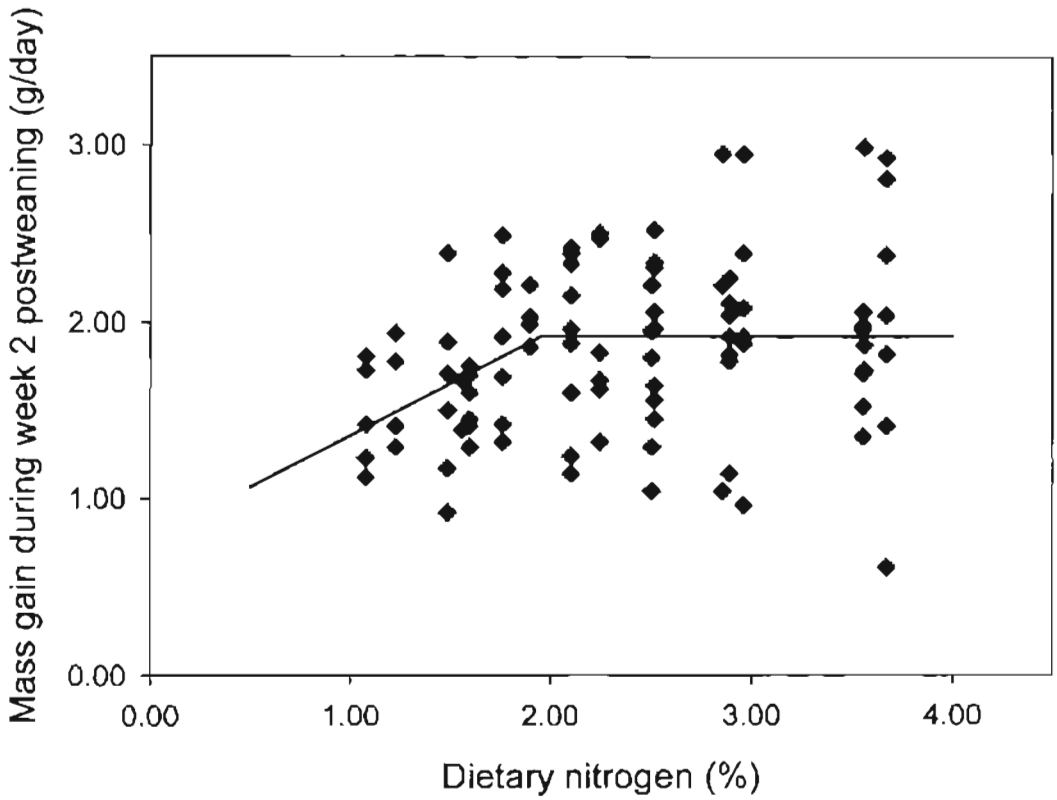
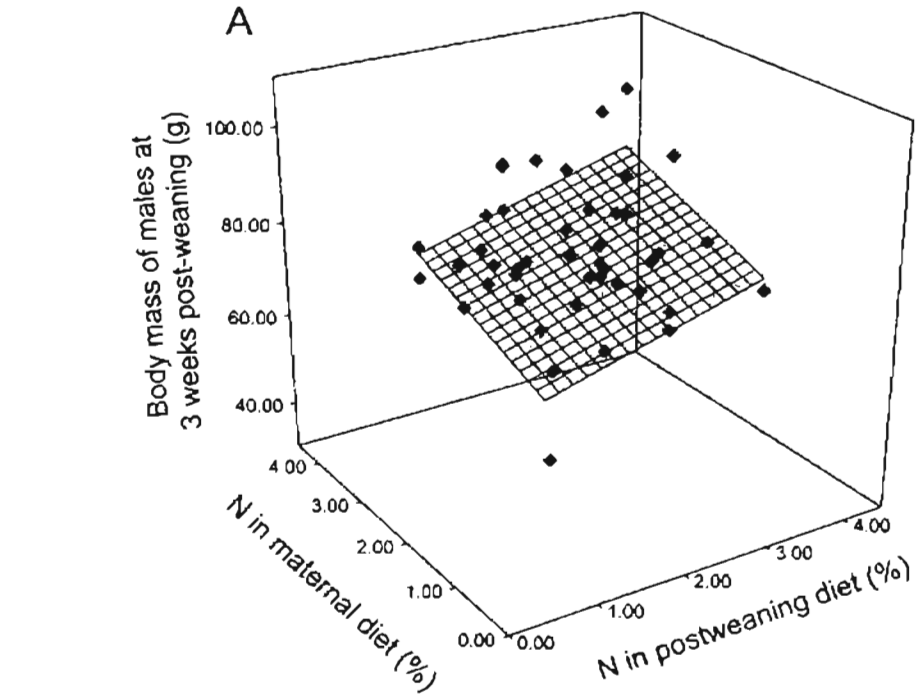
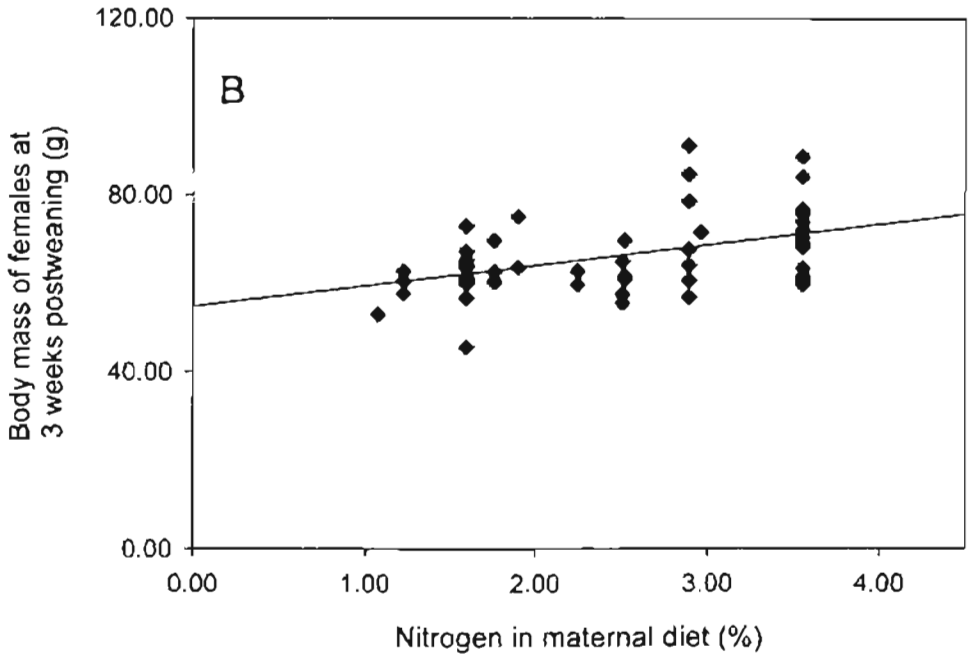

Mass gain during week 2 postweaning (g/day)
3.00
2.00
1.00
0.00
0.00
1.00
2.00
3.00
4.00
Dietary nitrogen (%)

Fig. 24.—A) Relationship between body mass at 3 weeks postweaning (g) and maternal (X_1) and postweaning (X_2) nitrogen intake (%) for male *S. hispidus* (n = 44; Y = 64.60 + $1.97X_1X_2$; $P < 0.001$; $r^2 = 0.26$). B) Relationship between body mass at 3 weeks postweaning (g) and maternal nitrogen intake (%) for female *S. hispidus* (n = 54; Y = 54.69 + 4.66X; $P < 0.001$; $r^2 = 0.20$).


A
Body mass of males at
3 weeks post-weaning (g)
100.00
80.00
60.00
40.00
4.00
3.00
2.00
1.00
0.00
N in maternal diet (%)
0.00
1.00
2.00
3.00
4.00
N in postweaning diet (%)


B
Body mass of females at
3 weeks postweaning (g)
120.00
80.00
40.00
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

body mass of females showed a linear relationship with % maternal nitrogen intake ($P < 0.001$; Fig. 24B; Table 11, Equation 36; Table 12). Six-week body mass (g) had a linear relationship with % maternal nitrogen intake for males ($P = 0.04$; Fig. 25; Table 11, Equation 37; Table 12) and with the square of maternal dietary nitrogen (%) for females ($P = 0.01$; Fig. 25; Table 11, Equation 38; Table 12), respectively. Body mass gained by males during the first 3 weeks after weaning showed a linear relationship with % postweaning nitrogen intake ($P = 0.01$; Fig. 26; Table 11, Equation 39; Table 12), whereas the same dependent variable for females was marginally related to both postweaning dietary nitrogen (%) and the square of postweaning diet ($P = 0.06$; Fig. 26; Table 11, Equation 40; Table 12).

Multiple regression analysis of growth-curve parameters showed no relationship ($P > 0.15$) between adult mass (g; Table 10) and any potential independent variable for offspring of captive-born *S. hispidus*. I also found that the response of time to adult mass (days; Table 10) to dietary nitrogen differed by maternal origin ($P = 0.03$). As a result, regression models were developed separately for offspring on captive-born and wild-caught females for those 2 dependent variables. The regression of maximum growth rate (g/day; Table 10) on nitrogen intake did not differ according to sex or maternal origin ($P \geq 0.61$).

The adult mass (g) of young born to wild-caught cotton rats was related to maternal nitrogen intake (%) and the interaction between maternal and postweaning diet ($P < 0.001$; Fig. 27; Table 11, Equation 41; Table 12). Time to adult mass (days) for young born to wild-caught rats responded to % dietary nitrogen postweaning, maternal dietary nitrogen (%), and the square of postweaning diet ($P < 0.001$; Fig. 28A; Table 11,

Fig. 25.—Relationship between body mass at 6 weeks postweaning (g) and maternal nitrogen intake (%) for *S. hispidus* (males, $n = 39$; $Y = 80.95 + 6.25X$; $P = 0.04$; $r^2 = 0.11$; females, $n = 44$; $Y = 74.21 + 1.25X^2$; $P = 0.01$; $r^2 = 0.13$).

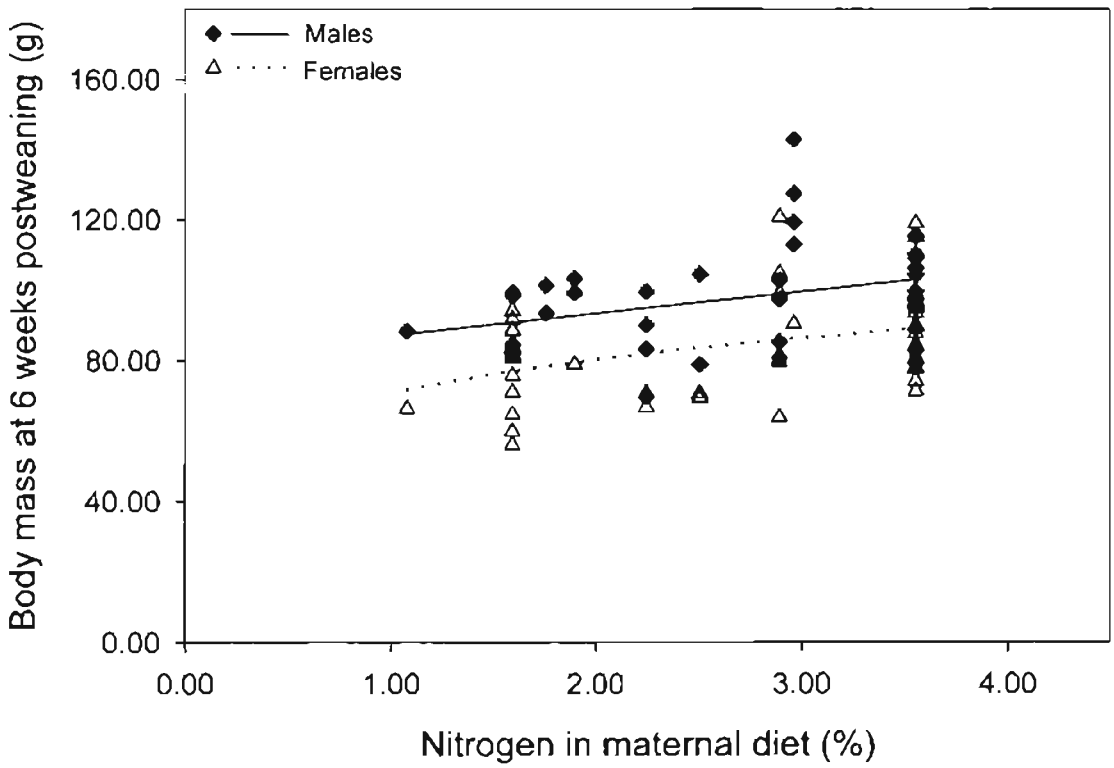

Males
Females
160.00
120.00
80.00
40.00
0.00
Body mass at 6 weeks postweaning (g)
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Fig. 26.—Relationship between body mass gained during the first 3 weeks postweaning (g) and postweaning nitrogen intake (%) for *S. hispidus* (males, $n = 44$, $Y = 32.37 + 4.41X$, $P = 0.01$, $r^2 = 0.17$; females, $n = 54$, $Y = 15.48 + 14.63X - 2.62X^2$, $P = 0.06$, $R^2 = 0.10$).

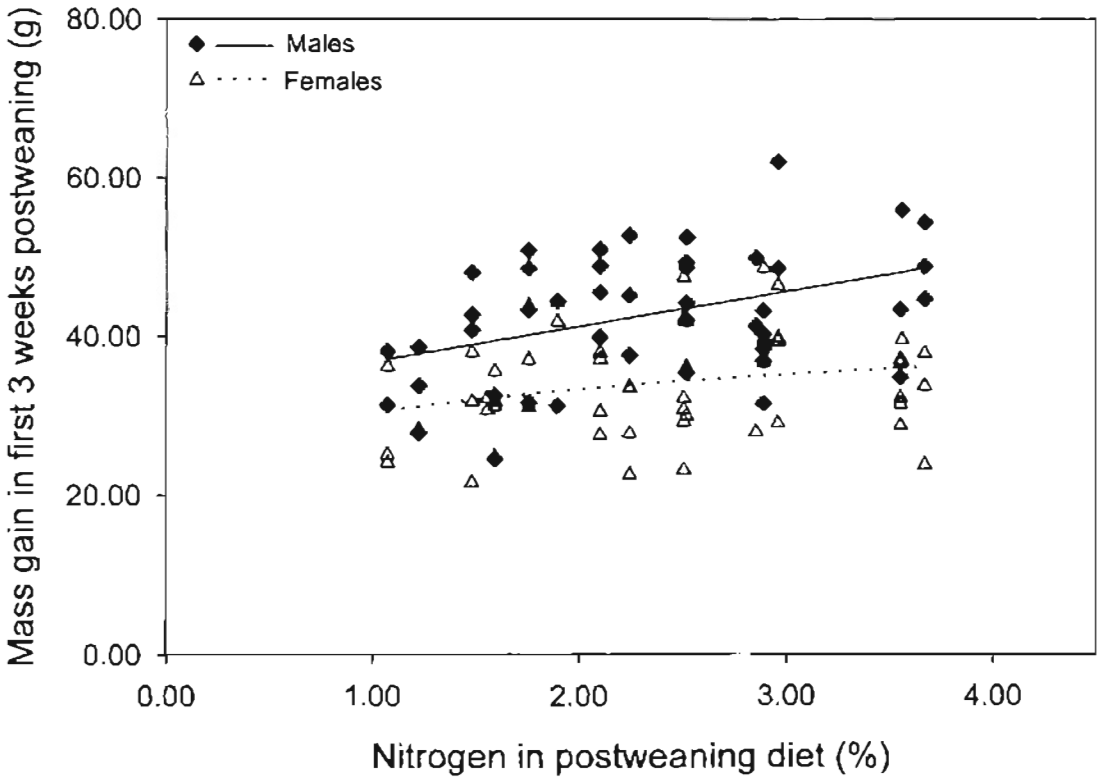

Males
Females
Mass gain in first 3 weeks postweaning (g)
80.00
60.00
40.00
20.00
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in postweaning diet (%)

Fig. 27.—Relationship between adult mass (g) and maternal (X_1) and postweaning (X_2) nitrogen intake (%) for postweanling *S. hispidus* born to wild-caught mothers ($n = 39$, $Y = 48.59 + 54.04X_1 - 8.92X_1X_2$, $P < 0.001$, $R^2 = 0.42$).

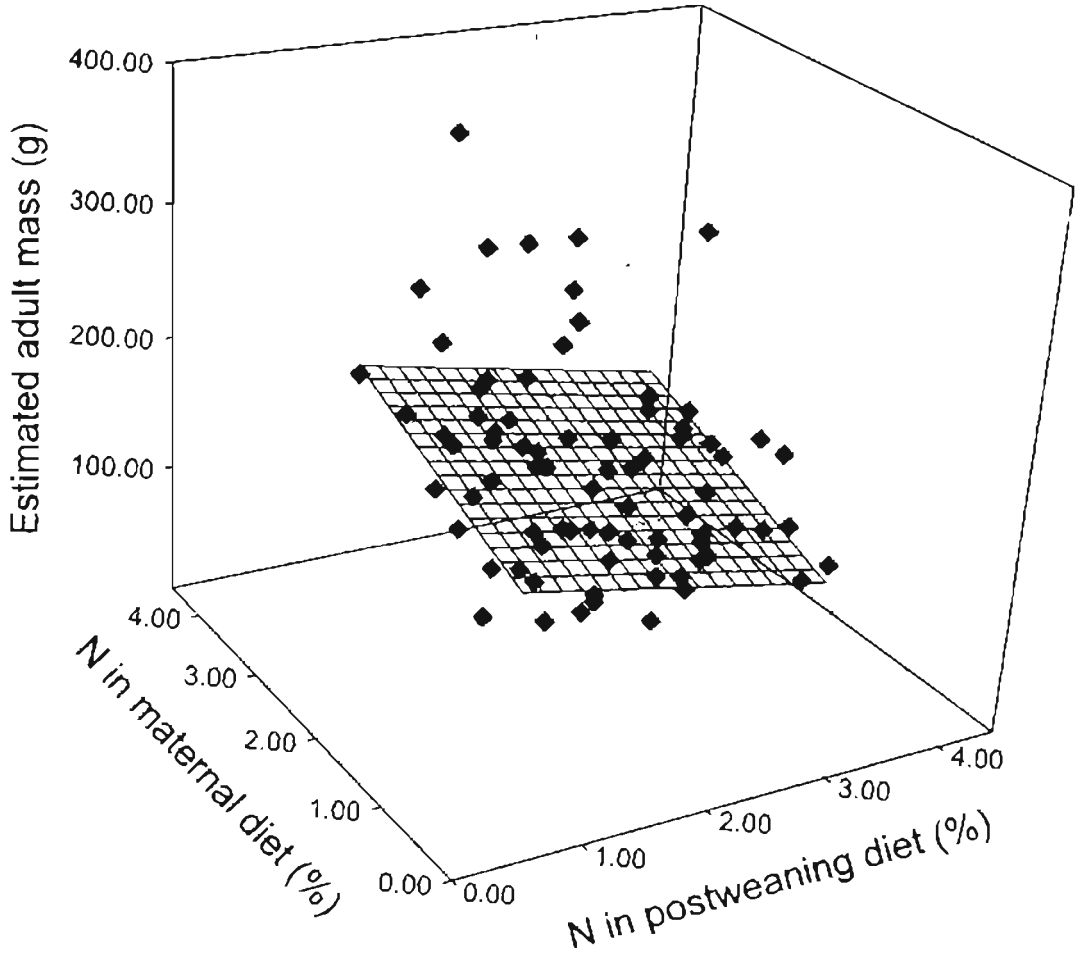
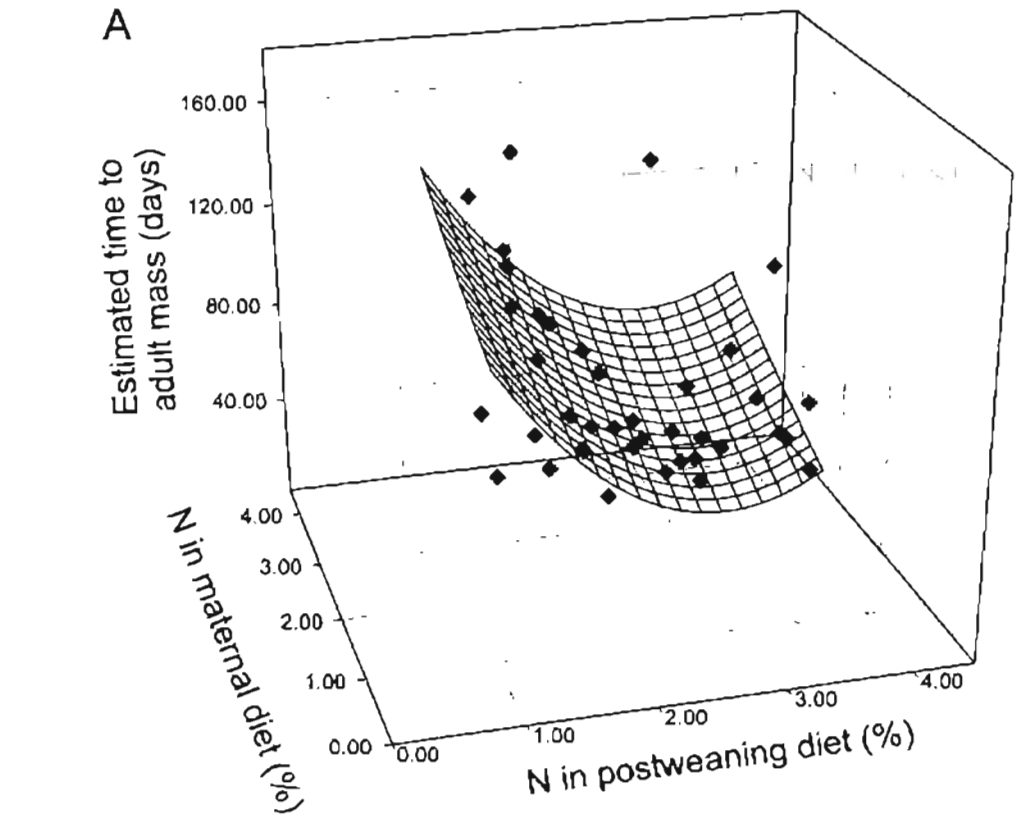
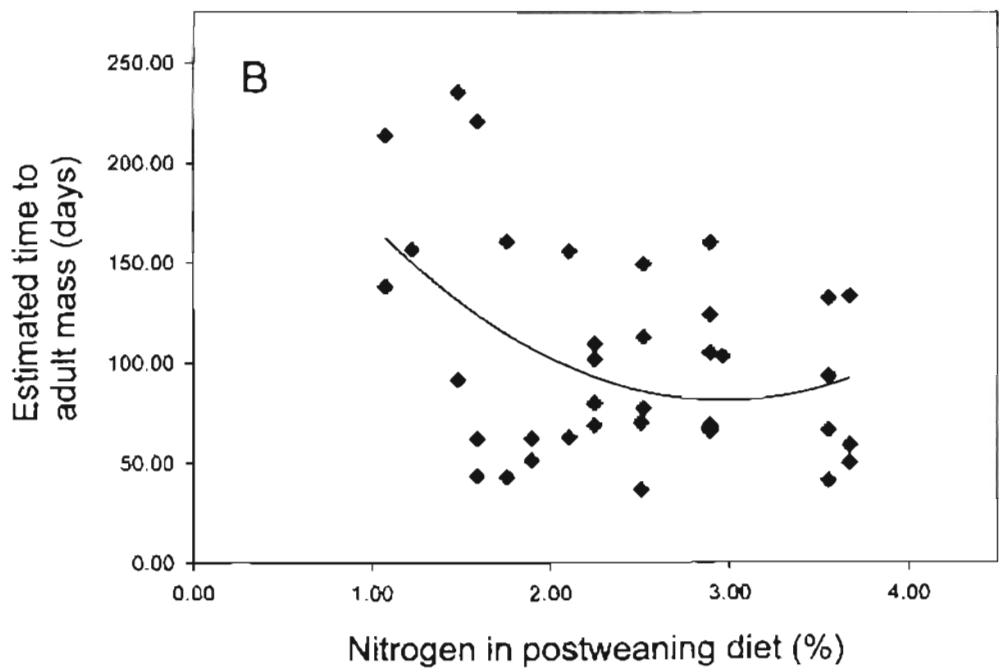

Estimated adult mass (g)
400.00
300.00
200.00
100.00
N in maternal diet (%)
4.00
3.00
2.00
1.00
0.00
N in postweaning diet (%)
0.00
1.00
2.00
3.00
4.00

Fig. 28.—A) Relationship between time to adult mass (days) and maternal (X_1) and postweaning (X_2) nitrogen intake (%) for postweanling *S. hispidus* born to wild-caught mothers ($n = 38$, $Y = 183.53 + 15.86X_1 - 111.28X_2 + 19.28X_2^2$, $P < 0.001$, $R^2 = 0.37$). B) Relationship between time to adult mass (days) and postweaning nitrogen intake (%) for postweanling *S. hispidus* born to captive-born mothers ($n = 39$, $Y = 280.88 - 134.51X + 22.62X^2$, $P = 0.02$, $R^2 = 0.19$).


A
Estimated time to adult mass (days)
160.00
120.00
80.00
40.00
N in maternal diet (%)
4.00
3.00
2.00
1.00
0.00
0.00
1.00
2.00
3.00
4.00
N in postweaning diet (%)


B
Estimated time to adult mass (days)
250.00
200.00
150.00
100.00
50.00
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in postweaning diet (%)

Equation 42; Table 12); the same dependent variable for offspring of captive-born rats was related to the linear and squared terms of % postweaning nitrogen intake ($P = 0.02$; Fig. 28B; Table 11, Equation 43; Table 12). Maximum growth rate for all cotton rats was related to % maternal dietary nitrogen and the interaction between maternal and postweaning diet ($P < 0.001$; Fig. 29; Table 11, Equation 44; Table 12).

Measurements of nitrogen conversion efficiency for cotton rats at week 1, 2, or 3 postweaning did not show any sex or maternal origin differences in the response of dependent variables to dietary nitrogen ($P \geq 0.39$; Table 10). Efficiency of nitrogen utilization (g daily gain in body mass per g daily nitrogen intake/$kg^{0.75}$) was linearly related to % postweaning nitrogen intake at 1 week ($P < 0.001$; Fig. 30; Table 11, Equation 45; Table 12) and 2 weeks ($P = 0.001$; Fig. 30; Table 11, Equation 46; Table 12) after weaning. Nitrogen utilization efficiency at week 3 postweaning showed a relationship with maternal nitrogen intake (%) and the interaction between postweaning and maternal diet ($P < 0.001$; Fig. 31; Table 11, Equation 47; Table 12).

I did not find a relationship ($P > 0.15$) between body mass (g) at week 6 postweaning and any potential independent variable for *R. fulvescens*, nor did I find a relationship for 3-week mass gain or the extrapolation of adult mass (Table 10) from weanling growth curves. Analysis of sex or maternal origin effects on multiple regression models showed a difference between males and females for week 3 body mass (g; $P < 0.001$); I therefore developed separate regression equations for this variable.

Body mass (g) of male harvest mice at 3 weeks after weaning responded to both the linear and squared terms of % maternal nitrogen intake ($P = 0.003$; Fig. 32; Table 11, Equation 48; Table 12). Week 3 mass (g) for female mice showed a weak negative linear

Fig. 29.—Relationship between maximum growth rate (g/day) and maternal (X_1) and postweaning (X_2) nitrogen intake (%) for postweanling *S. hispidus* ($n = 79$, $Y = 2.80 - 0.55X_1 + 0.08X_1X_2$, $P < 0.001$, $R^2 = 0.24$).

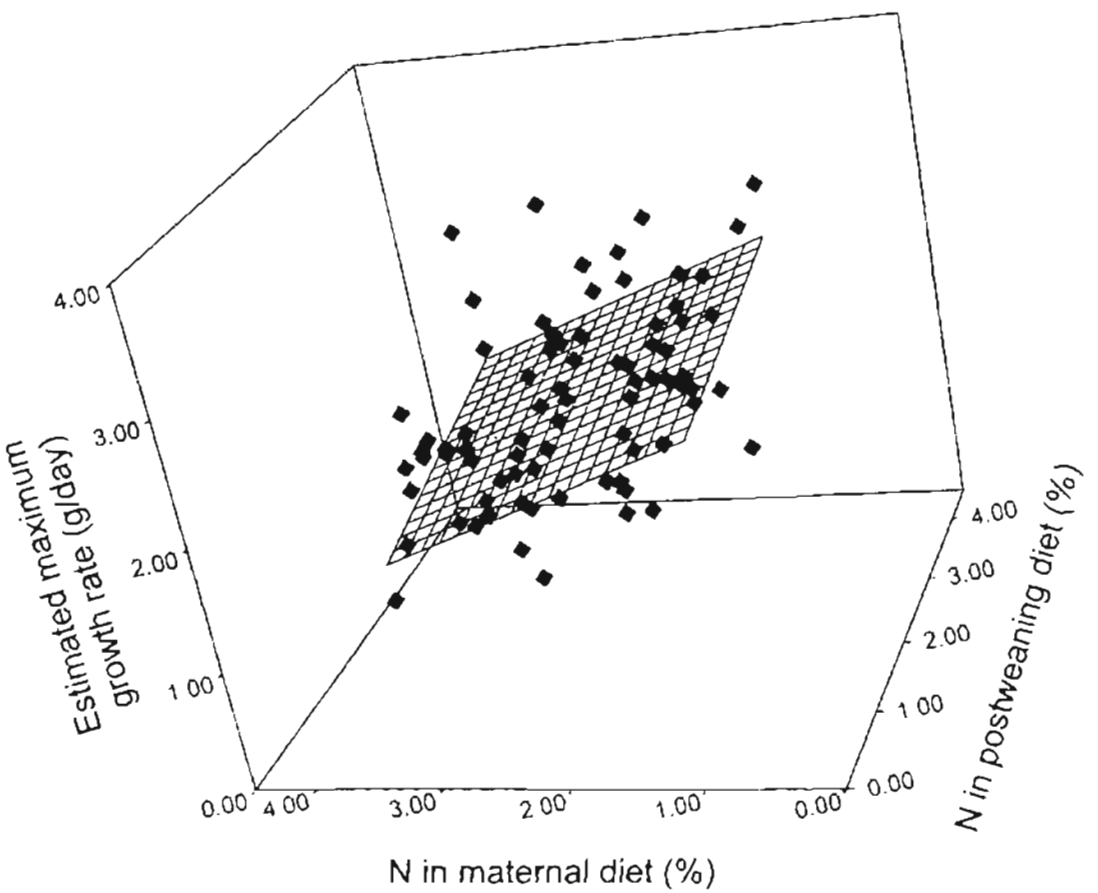

Estimated maximum growth rate (g/day)
4.00
3.00
2.00
1.00
0.00
4.00
3.00
2.00
1.00
0.00
N in maternal diet (%)
N in postweaning diet (%)
4.00
3.00
2.00
1.00
0.00

Fig. 30.—Relationship between nitrogen utilization efficiency at 1 and 2 weeks postweaning (g daily gain in body mass per g daily nitrogen intake/$kg^{0.75}$) and postweaning nitrogen intake (%) for *S. hispidus* (week 1, $n = 37$, $Y = 2.10 - 0.36X$, $P < 0.001$, $r^2 = 0.46$; week 2, $n = 26$, $Y = 1.94 - 0.34X$, $P = 0.001$, $r^2 = 0.36$).

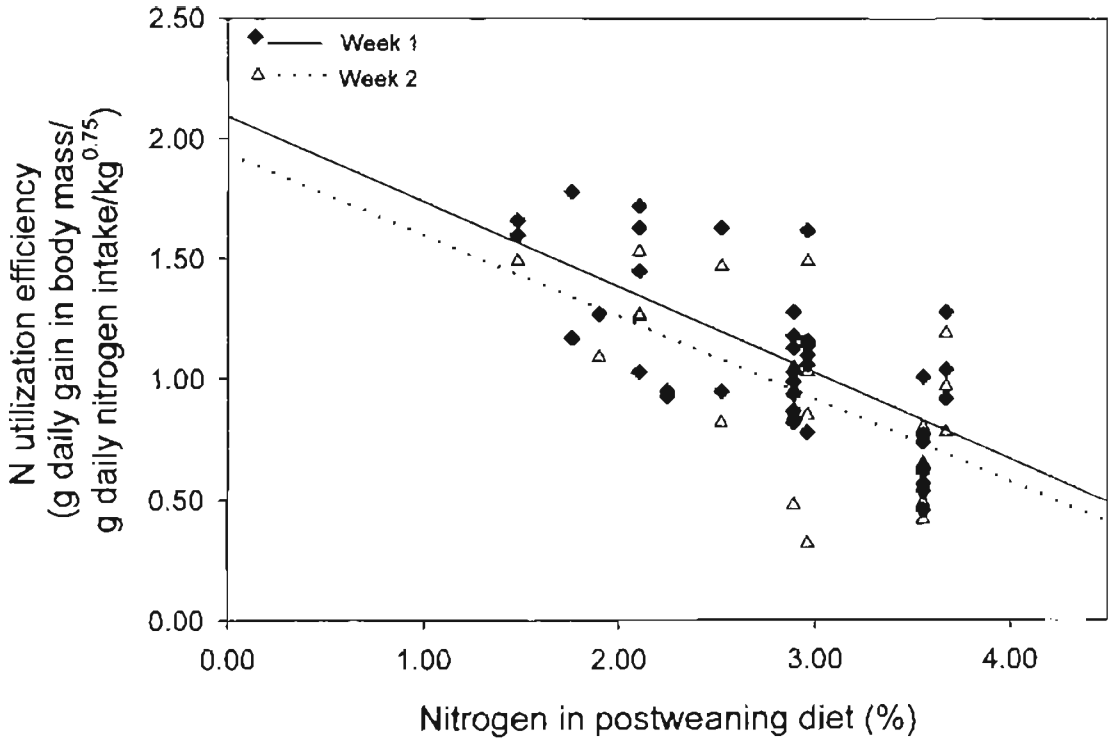

Week 1
Week 2
N utilization efficiency
(g daily gain in body mass/
g daily nitrogen intake/kg$^{0.75}$)
2.50
2.00
1.50
1.00
0.50
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in postweaning diet (%)

Fig. 31.—Relationship between nitrogen utilization efficiency at 3 weeks postweaning (g daily gain in body mass per g daily nitrogen intake/$kg^{0.75}$) and maternal (X_1) and postweaning (X_2) nitrogen intake (%) for *S. hispidus* ($n = 25$, $Y = 0.37 + 0.48X_1 - 0.10X_1X_2$, $P < 0.001$, $R^2 = 0.47$).

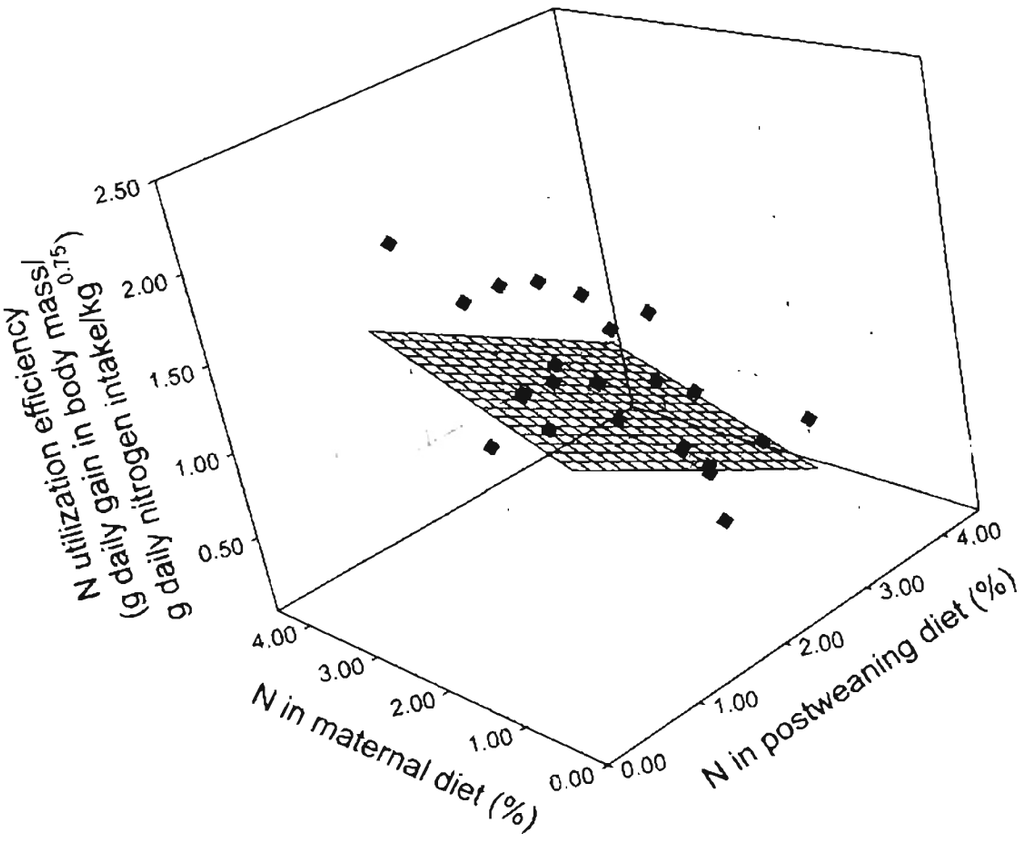
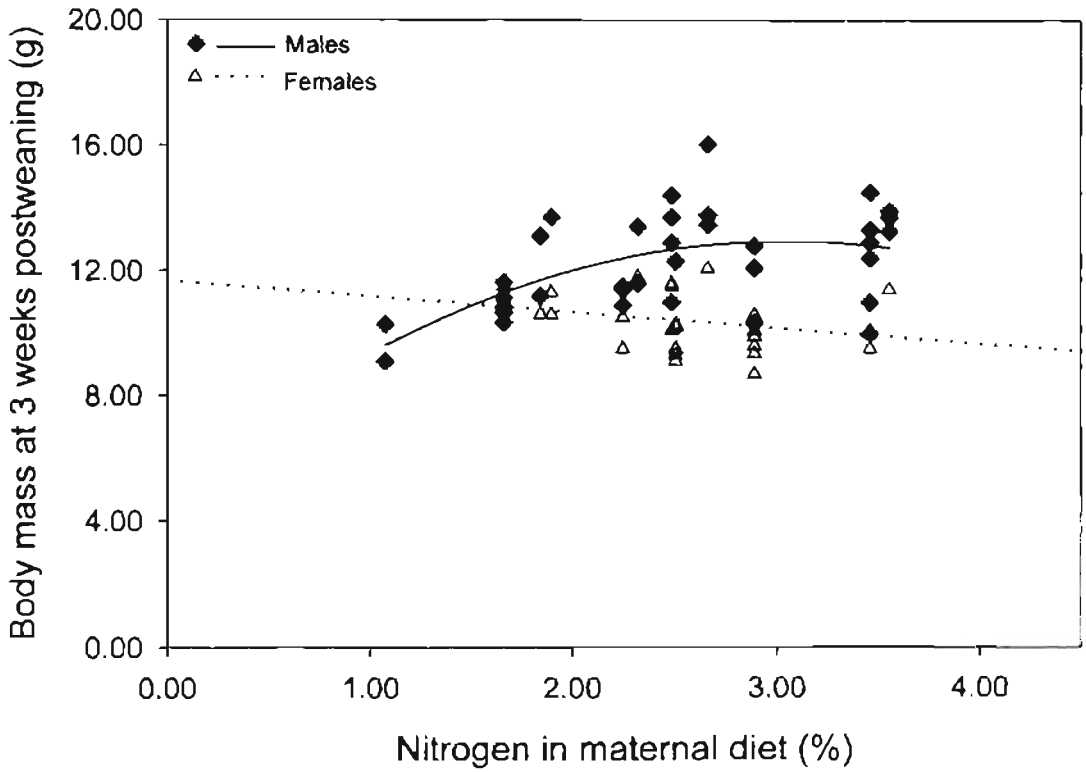

N utilization efficiency
(g daily gain in body mass$_{0.75}$)
g daily nitrogen intake/kg
2.50
2.00
1.50
1.00
0.50
4.00
3.00
2.00
1.00
0.00
N in maternal diet (%)
0.00
1.00
2.00
3.00
4.00
N in postweaning diet (%)

Fig. 32.—Relationship between body mass at 3 weeks postweaning (g) and maternal nitrogen intake (%) for *R. fulvescens* (males, $n = 35$, $Y = 5.00 + 5.20X - 0.85X^2$, $P = 0.003$, $R^2 = 0.31$; females, $n = 34$, $Y = 11.70 - 0.51X$, $P = 0.08$, $r^2 = 0.09$).


Males
Females
Body mass at 3 weeks postweaning (g)
20.00
16.00
12.00
8.00
4.00
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

relationship with % maternal dietary nitrogen ($P = 0.08$; Fig. 32; Table 11, Equation 49; Table 12). Analysis of time to adult mass (days; Table 10) for all harvest mice indicated a response to the square of postweaning dietary nitrogen (%), the square of maternal dietary nitrogen (%), and the interaction between maternal and postweaning nitrogen intake ($P = 0.04$; Fig. 33; Table 11, Equation 50; Table 12). The estimate of maximum growth rate (g/day) from individual growth curves had a marginal negative relationship with % maternal nitrogen intake ($P = 0.05$; Fig. 34; Table 11, Equation 51; Table 12).

The response of nitrogen utilization efficiency at week 2 postweaning to dietary nitrogen varied marginally ($P = 0.06$) by sex for *R. fulvescens* (Table 10). I also found an effect of maternal origin on the relationship between nitrogen utilization efficiency during week 3 (Table 10) and nitrogen intake ($P = 0.03$), with offspring of wild-caught females showing a negative relationship ($P < 0.001$) with the square of postweaning diet, whereas young of captive-born females showed a marginal positive relationship ($P = 0.12$) with % postweaning nitrogen intake. However, only 9 of the 34 animals with accurate nitrogen conversion measurements were born to captive-born females. As a result, separate regression models were developed for nitrogen efficiency at week 2, but not at week 3 postweaning.

Nitrogen utilization (g daily gain in body mass per g daily nitrogen intake/$kg^{0.75}$) of all harvest mice at 1 week after weaning (Table 10) was negatively related to % maternal nitrogen intake and % postweaning dietary nitrogen ($P < 0.001$; Fig. 35; Table 11, Equation 52; Table 12). Nitrogen conversion efficiency (g gain/g nitrogen intake/$kg^{0.75}$) for males during week 2 was related to the square of % postweaning dietary nitrogen ($P = 0.002$; Fig. 36A; Table 11, Equation 53; Table 12), and for females was

Fig. 33.—Relationship between time to adult mass (days) and maternal (X_1) and postweaning (X_2) nitrogen intake (%) for postweanling *R. fulvescens* ($n = 64$, $Y = 45.81 + 1.43X_1^2 + 2.15X_2^2 - 3.57X_1X_2$, $P = 0.04$, $R^2 = 0.13$).

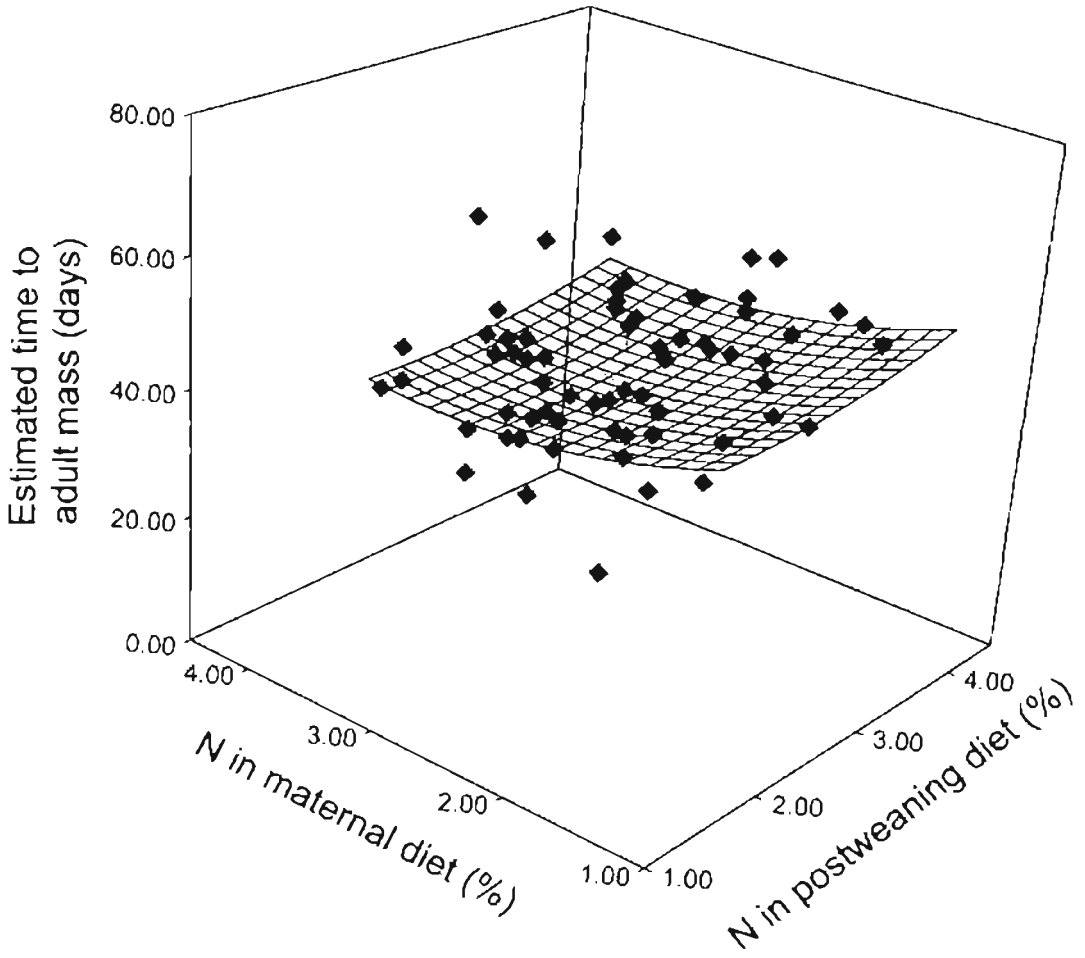

Estimated time to adult mass (days)
80.00
60.00
40.00
20.00
0.00
4.00
3.00
2.00
1.00
N in maternal diet (%)
1.00
2.00
3.00
4.00
N in postweaning diet (%)

Fig. 34.—Relationship between maximum growth rate (g/day) and maternal nitrogen intake (%) for postweanling *R. fulvescens* ($n = 64$, $Y = 0.41 - 0.04X$, $P = 0.05$, $r^2 = 0.06$).

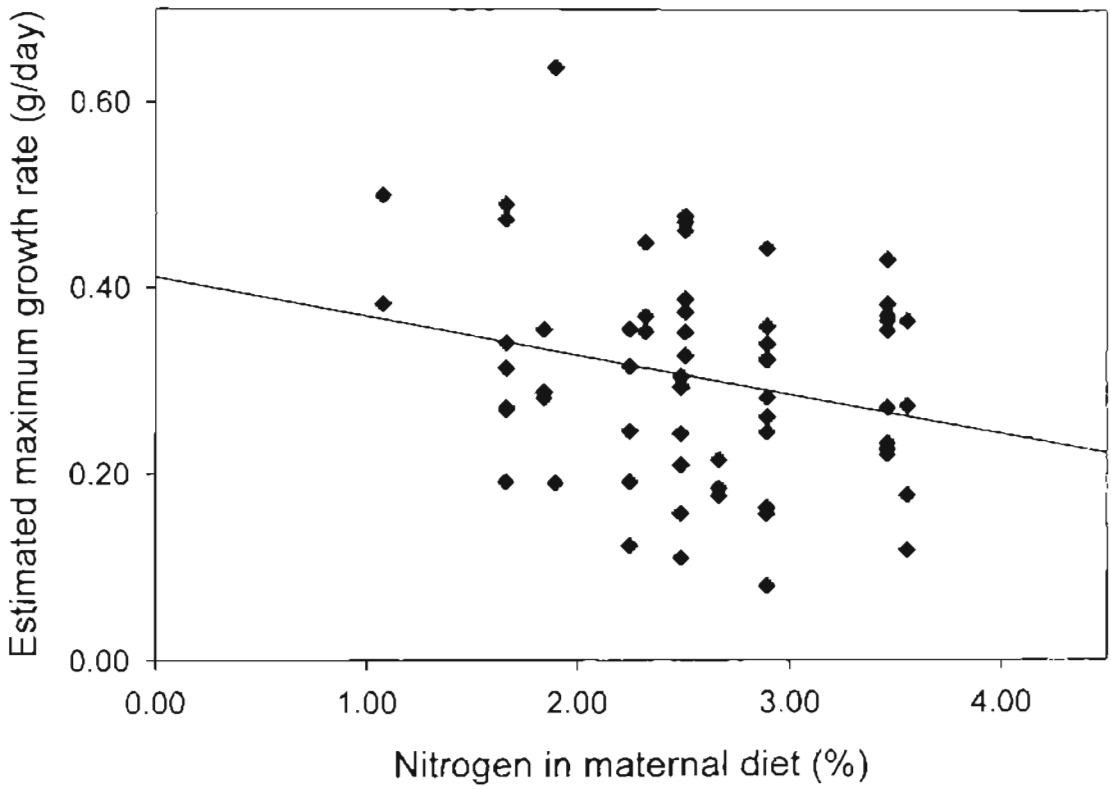

Estimated maximum growth rate (g/day)
0.60
0.40
0.20
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in maternal diet (%)

Fig. 35.—Relationship between nitrogen utilization efficiency at 1 week postweaning (g daily gain in body mass per g daily nitrogen intake/$kg^{0.75}$) and maternal (X_1) and postweaning (X_2) nitrogen intake (%) for *R. fulvescens* ($n = 52$, $Y = 0.20 - 0.01X_1 - 0.03X_2$, $P < 0.001$, $R^2 = 0.48$).

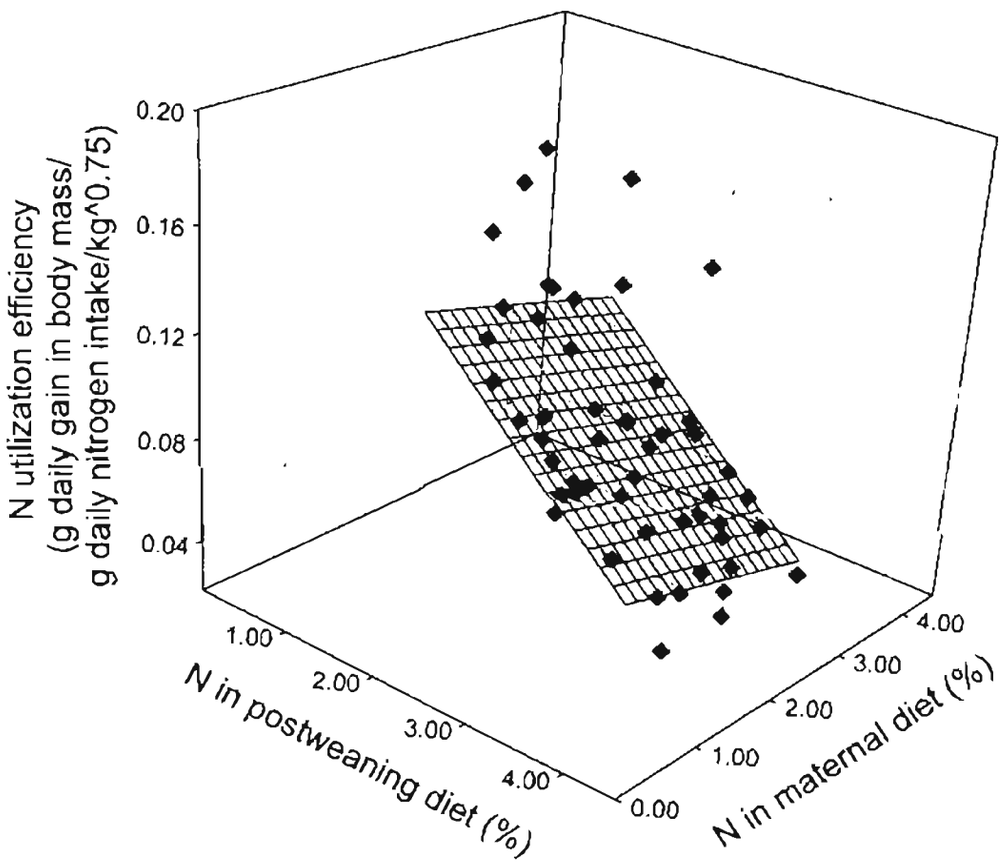
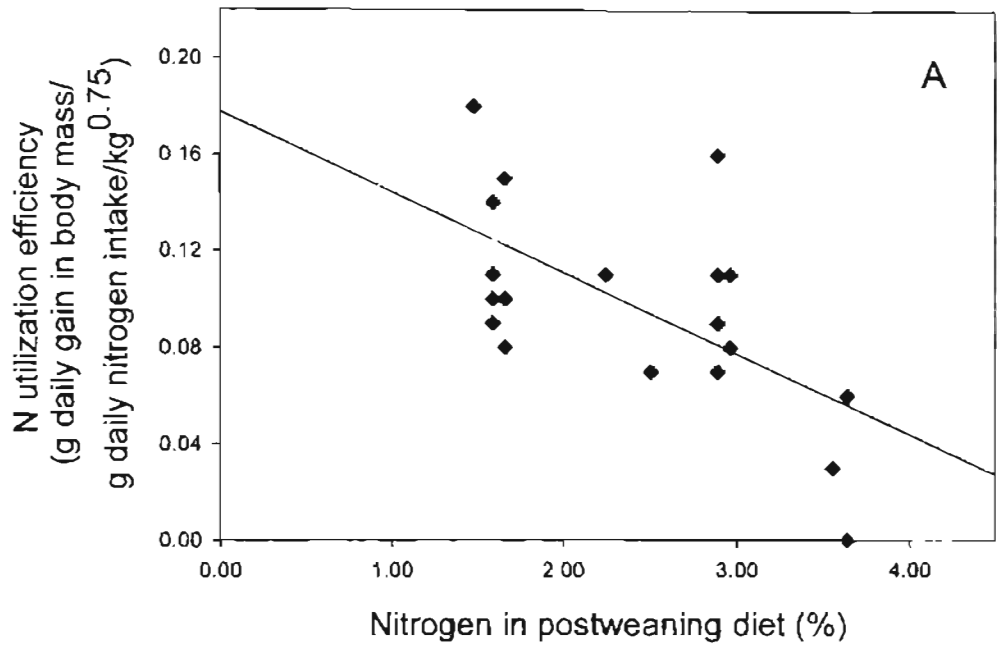
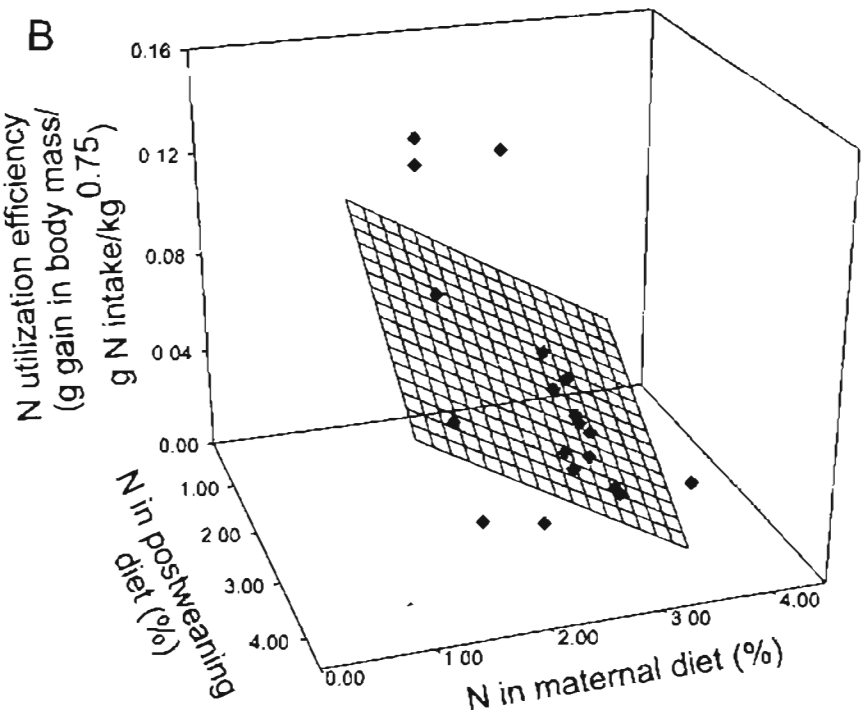

N utilization efficiency
(g daily gain in body mass/
g daily nitrogen intake/kg^0.75)
0.20
0.16
0.12
0.08
0.04
1.00
2.00
3.00
4.00
N in postweaning diet (%)
0.00
1.00
2.00
3.00
4.00
N in maternal diet (%)

Fig. 36.—A) Relationship between nitrogen utilization efficiency at 2 weeks postweaning (g daily gain in body mass per g daily nitrogen intake/$kg^{0.75}$) and postweaning nitrogen intake (%) for male *R. fulvescens* ($n = 20$, $Y = 0.14 - 0.01X^2$, $P = 0.002$, $r^2 = 0.43$). B) Relationship between nitrogen utilization efficiency at 2 weeks postweaning (g daily gain in body mass per g daily nitrogen intake/$kg^{0.75}$) and maternal (X_1) and postweaning (X_2) nitrogen intake (%) for female *R. fulvescens* ($n = 19$, $Y = 0.11 - 0.01X_1X_2$, $P = 0.002$, $r^2 = 0.43$).


A
N utilization efficiency
(g daily gain in body mass/
g daily nitrogen intake/kg$^{0.75}$)
0.20
0.16
0.12
0.08
0.04
0.00
0.00
1.00
2.00
3.00
4.00
Nitrogen in postweaning diet (%)


B
N utilization efficiency
(g gain in body mass/
g N intake/kg$^{0.75}$)
0.16
0.12
0.08
0.04
0.00
N in postweaning diet (%)
1.00
2.00
3.00
4.00
0.00
1.00
2.00
3.00
4.00
N in maternal diet (%)

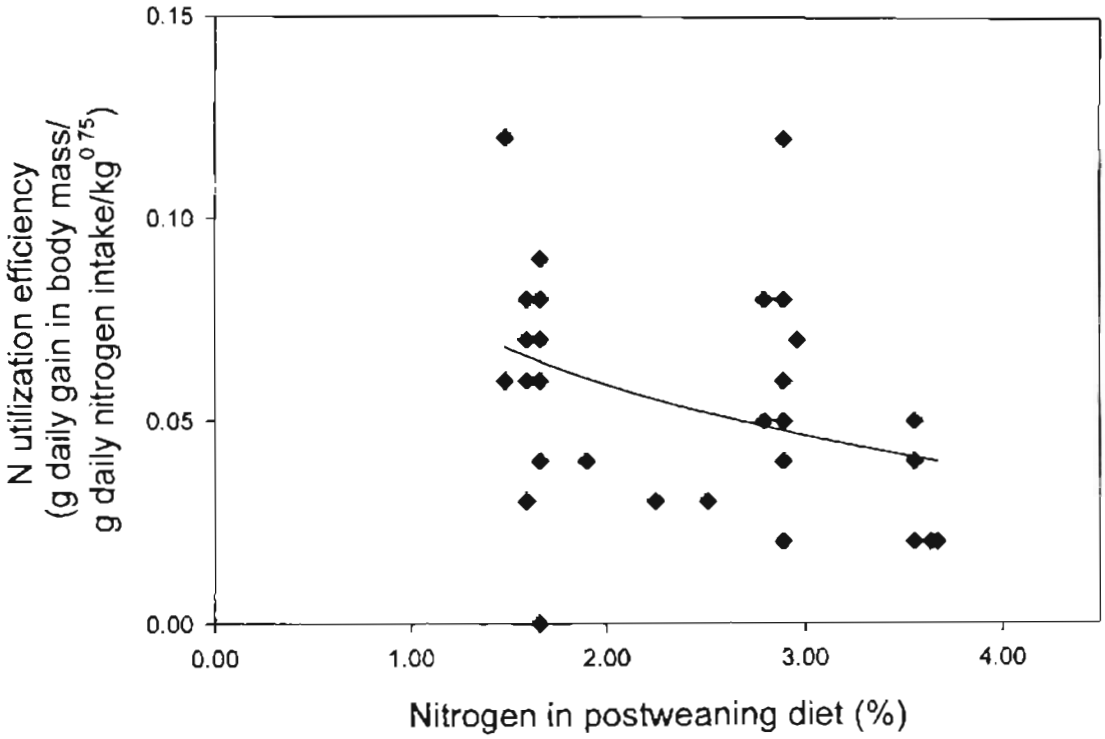
related to the interaction between % maternal and postweaning nitrogen intake (P = 0.002; Fig. 36B; Table 11, Equation 54; Table 12). Finally, efficiency of nitrogen utilization (g gain/g nitrogen intake/kg$^{0.75}$) for all mice at 3 weeks after weaning was related to the square of % postweaning dietary nitrogen (P = 0.02; Fig. 37; Table 11, Equation 55; Table 12).

Interspecies Comparisons

A factorial (species by sex) ANOVA of mean weanling DMI (g/kg$^{0.75}$/day) found no within-species sex differences and no interactions of the 2 variables ($P \geq 0.12$) at 1, 2, and 3 weeks after weaning. DMI varied by species during each week (n = 97, 66, and 59; all $P < 0.001$), with *R. fulvescens* showing consistently higher intake rates (Table 10). The same analysis of efficiencies of nitrogen utilization (g daily gain/g daily nitrogen intake/kg$^{0.75}$) indicated an interaction at 1 week after weaning (n = 87, P = 0.02), with consistent species differences ($P < 0.001$) and within-species sex differences for *S. hispidus* (P = 0.002) but not for *R. fulvescens* (P = 0.86); cotton rats had at least 10-fold higher nitrogen conversion rates at week 1 than harvest mice, and male rats utilized dietary nitrogen more efficiently than female rats (Table 10). Nitrogen conversion efficiency at weeks 2 and 3 did not have any sex effects or interactions (n = 63 and 57; $P \geq 0.10$), but there was a species effect ($P < 0.001$), with cotton rats again showing higher utilization efficiencies by an order of magnitude (Table 10).

Because growth rate data for *R. fulvescens* did not fit breakpoint models during any of the first 3 weeks after weaning, I was unable to carry out species comparisons of dietary requirements for optimum postweaning growth. However, I did perform factorial ANOVAs of adult mass (g), time to adult mass (days), and maximum growth rate (g/day)

Fig. 37.—Relationship between nitrogen utilization efficiency at 3 weeks postweaning (g daily gain in body mass per g daily nitrogen intake/$kg^{0.75}$) and postweaning nitrogen intake (%) for *R. fulvescens* ($n = 34$, $Y = 0.07 - 0.003X^2$, $P = 0.02$, $r^2 = 0.16$).


0.15
0.10
0.05
0.00
0.00
1.00
2.00
3.00
4.00
N utilization efficiency
(g daily gain in body mass/
g daily nitrogen intake/kg$^{0.75}$)
Nitrogen in postweaning diet (%)

as calculated from individual growth curves. This analysis showed a main effect of species for all variables ($n = 141$, $P < 0.001$). Cotton rats had larger body masses, took almost twice as long to reach their adult mass, and showed higher maximum growth rates by a factor of 6 (Table 10). There was also a difference by sex approaching significance ($P = 0.08$) for adult mass, with males reaching larger body masses than females for both species.

Effects of species and sex interacted on rates of gain for cotton rats and harvest mice during the first 3 weeks postweaning and for daily rates of gain at 1, 2, and 3 weeks after weaning ($n = 167$, $P \leq 0.02$). In all cases, there were species effects across both sexes ($P < 0.001$) and within-species sex differences for *S. hispidus* ($P < 0.001$), but not for *R. fulvescens* ($P \geq 0.20$). Cotton rats showed postweaning growth rates that were 10 times higher than those of harvest mice (Table 10), and male rats grew substantially faster than female rats.

DISCUSSION

Maintenance Requirements

General

The lack of a relationship between DMI (g/kg$^{0.75}$/day) and dietary nitrogen (%) or DDMI (g/kg$^{0.75}$/day) and nitrogen intake (mg) for cotton rats and harvest mice indicates that animals undergoing maintenance feeding trials were not using compensatory intake to alter their diet. Therefore, it follows that they consumed similar amounts and proportions of nitrogen as those offered in experimental rations. Furthermore, a slope equal to 1 and an intercept equal to 0 for the relationship between ort nitrogen (%) and dietary nitrogen (%) in *R. fulvescens* demonstrates a lack of selection for higher or lower-nitrogen portions of the diet. In *P. leucopus*, a slope > 1 and intercept > 0 for the same regression indicated some capacity for selective intake in this species. However, there was a difference of only 4 – 11 % between nitrogen offered to white-footed mice and nitrogen consumed. I feel that the statistical significance of this analysis may be attributed to large sample size but does not represent a biologically significant difference.

Although DMI did not generally change with nitrogen intake, it should be noted that the experimental rations used in this study were formulated for equality of energy content. Evidence suggests that after minimum requirements of diet quality are met so that physical limitations (gastrointestinal capacity, passage rate) do not impede intake rates, digestible energy intake is the principal cue for physiological regulation of feeding rates (Batzli and Cole 1979; Karasov 1982; Robbins 1993; Pond et al. 1995). Under natural conditions, nitrogen and energy content are positively correlated in most

foodstuffs (Cheeke 1991; Robbins 1993; Pond et al. 1995), and therefore a negative relationship between nitrogen and DMI may exist under free-ranging conditions.

Estimates of nitrogen flux

Values of MFN for all genera in this study were generally below those reported in previous studies on rodents (Table 13). Coprophagy greatly reduces fecal nitrogen losses in other species (Chilcott and Hume 1984; Robbins 1993), and it is possible that my study species made use of this mechanism to preserve ingested nitrogen. Cotton rats practice coprophagy (J. L. Parsons, pers. obs.), and morphology of the hindgut of all 3 mouse species indicates some cecotrophic capacity (J. L. Parsons, pers. obs.). Some measurements of MFN were made on animals that were consuming rations with higher fiber content or antiherbivore compounds that would induce much higher fecal nitrogen losses (Table 3; Meyer 1956; Robbins et al. 1987; Kam and Degen 1988; van Zyl et al. 1999; Felicetti et al. 2000). Furthermore, previous studies involved a number of desert-dwelling species (Karasov 1982; Kam and Degen 1988; Meyer and Karasov 1989). Jackson and Spinks (1998) found that arid-zone rodents have longer large intestines than their mesic counterparts, providing more mucosal surface area and therefore more opportunity for endogenous nitrogen loss.

Estimated values of EUN for *S. hispidus* and *P. leucopus* fall within the range of values observed in other species (Table 14). Although EUN for white-footed mice is higher than reported values for other rodents, previous work was conducted on desert-dwelling species (e.g., antelope ground squirrel, fat sand rat) or herbivores (e.g., guinea pig). The magnitude of EUN is closely related to basal metabolic rate (Smuts 1935;

Table 13.—Previously reported values of metabolic fecal nitrogen (MFN) for various mammalian species.

Species	Diet	MFN (g N/ kg DMI)	Reference
Marsupials			
Sugar glider	Honey and pollen	0.70	Smith and Green 1987
Brushtail possum	Honey and low to high fiber	0.78-3.36	Wellard and Hume 1981
Ringtail possum	Eucalyptus leaves	2.38	Chilcott and Hume 1984
Quokka	Forage	2.90	Brown 1968
Koala	Eucalyptus leaves	5.90	Cork 1986
Rufous rat-kangaroo	Grain and oat hulls	3.20	Robbins 1993
Parma wallaby	Forage	5.70	Hume 1986
Tammar wallaby	Forage and concentrates	4.10	Brown 1968
Kangaroo Island wallaby	Concentrates	4.11	Barker 1968
Euro	Forage and concentrates	2.70-3.24	Brown 1968; Robbins 1993
Red kangaroo	Forage	4.73	Hume 1974
Average		**3.37**	
Ruminants			
Hartebeest	Concentrates	4.16	Robbins 1993
Bison	Forage	4.14	Robbins 1993
Yak	Forage	4.57	Robbins 1993
Eland	Concentrates	3.53	Robbins 1993
Nilgai antelope	Concentrates	5.66	Priebe and Brown 1987
Red deer	Forage and concentrates	6.98	Robbins 1993

Table 13 (continued).

Species	Diet	MFN (g N/ kg DMI)	Reference
Ruminants (continued)			
Thomson's gazelle	Concentrates	4.77	Robbins 1993
Caribou/reindeer	Forage and concentrates	6.26	Robbins et al. 1987
Elk	Forage	5.58	Mould and Robbins 1981
Nubian ibex	Forage	5.17	Robbins 1993
Bush duiker	Concentrates	3.63	Robbins 1993
Moose	Concentrates and sawdust	5.06	Robbins et al. 1987; Schwartz et al. 1987
White-tailed and mule deer	Concentrates	6.38	Robbins et al. 1987
	Forage	6.19	Robbins et al. 1987
White-tailed deer	Concentrates	2.58-5.15	Asleson et al. 1996
Average		**4.99**	
Preruminant cervids			
Elk	Milk	1.26	Robbins 1993
White-tailed deer	Milk	2.37	Robbins 1993
Nonruminant eutherians			
Rhesus monkey	Concentrates	1.04	Robbins and Gavan 1966
Bears (black, grizzly, giant panda)	Forage, berries, nuts, or tubers	5.54	Robbins 1993
Collared peccary	Concentrates and forage	4.52	Carl and Brown 1985
Rock hyrax	Concentrates	3.69	Hume et al. 1980

Table 13 (concluded).

Species	Diet	MFN (g N/ kg DMI)	Reference
Nonruminant eutherians (continued)			
Black-tailed jackrabbit	Forage	4.61	Nagy et al. 1976
Domestic rabbit	Forage	8.00	Robbins 1993
Snowshoe hare	Semisynthetic	6.10	Holter et al. 1974
North American porcupine	Semisynthetic or fruits, nuts, oats	0.92	Fournier and Thomas 1997
	Natural forages	2.80	Felicetti et al. 2000
Desert woodrat	Concentrates	3.18	Meyer and Karasov 1989
	Forage	8.10	Meyer and Karasov 1989
Antelope ground squirrel	Semisynthetic	2.00	Karasov 1982
Fat sand rat	Saltbush	70.5 mg/ $kg^{0.75}$/day	Kam and Degen 1988
Average		**4.21**	
Hispid cotton rat		1.72	This study
Fulvous harvest mouse		2.00	This study
White-footed mouse		1.73	This study

Table 14.— Previously reported values of endogenous urinary nitrogen (EUN) for various mammalian species.

Species	EUN ($mg/kg^{0.75}/day$)	Reference
Marsupials		
Ringtail possum	191	Chilcott and Hume 1984
Quokka	43	Brown 1968
Black-footed rock wallaby	49	Brown 1968
Tammar wallaby	58	Barker 1968
Parma wallaby	108	Hume 1986
Euro	32	Brown 1968
Red kangaroo	87	Brown 1968
Red-necked pademelon	80	Hume 1977
Rufous rat-kangaroo	46	Robbins 1993
Hairy-nosed wombat	42	Barboza et al. 1993
Common wombar	31	Barboza et al. 1993
Greater glider	200	Foley and Hume 1987
Sugar glider	25	Smith and Green 1987
Koala	51	Harrop and Degabriele 1976; Cork 1986
Average	**75**	
Ruminants		
Elk	160	Mould and Robbins 1981
Moose	56	Schwartz et al. 1987
Red deer	90	Robbins 1993
Roe deer	78	Eisfeld 1974

Table 14 (concluded).

Species	EUN ($mg/kg^{0.75}/day$)	Reference
Ruminants (continued)		
White-tailed deer	115-145	Robbins 1993; Asleson et al. 1996
Domestic goat	115	Hume 1999
Domestic sheep	87	Smuts and Marais 1938
Dromedary camel	60	Robbins 1993; Hume 1999
Average	**97**	
Nonruminant eutherians		
Collared peccary	177	Carl and Brown 1985
Black-tailed jackrabbit	128	Nagy et al. 1976
Domestic rabbit	148	Hume 1999
North American porcupine	223	Fournier and Thomas 1997
	205	Felicetti et al. 2000
Antelope ground squirrel	163	Karasov 1982
Fat sand rat	171	Kam and Degen 1988
Guinea pig	145	Hume 1999
Average	**153**	
Hispid cotton rat	158	This study
Fulvous harvest mouse	348	This study
White-footed mouse	237	This study

Karasov 1982; Wallis and Hume 1992; Robbins 1993; Hume 1999). Generally, animals inhabiting xeric environments have lower metabolic rates (McNab 1989; Yahav and Choshniak 1989; Merkt and Taylor 1994; Rubal et al. 1995; Kronfeld and Shkolnik 1996; Hume 1999; Lovegrove 2000) and consequently lower rates of nitrogen flux. In addition, herbivores and arid-zone species show lower levels of urinary nitrogen excretion, due to the recycling of urea from the bloodstream into the gastrointestinal tract for microbial fermentation or nitrogen conservation (Hume et al. 1980; Kam and Degen 1988; Yahav and Chosniak 1989; Campbell and MacArthur 1997; Hume 1999). Therefore, I believe that the higher estimate of EUN in *P. leucopus* may be attributed to differences in native habitat and diet preference.

I discovered a lower body-size limit when feeding animals in the type of metabolic cage I was using. Because of their small size (10-15 g), *R. fulvescens* excreted little urine, and much of the urine excreted by some individuals tended to remain on the plastic surface of the collection funnel and evaporate. As a result, I relied exclusively on cage washing to collect nitrogen of urinary origin for harvest mice. Although I was careful to use centrifugation to separate the liquid portion of cage washes from any spilled food that was washed into collection vials, I suspect that some leaching of nitrogen from food spillage into the supernatant occurred. Therefore, I modeled urinary nitrogen excretion, assuming that the same relationship between urinary and fecal nitrogen excretion holds for harvest mice as it does for cotton rats.

I performed a regression of the ratio between urinary and fecal nitrogen excretion on nitrogen intake ($mg/kg^{0.75}/day$) for *S. hispidus* and *P. leucopus* combined (Table 7, Equation 81). Using the estimated equation of this regression and experimentally

obtained data on *R. fulvescens* fecal excretion rates, I predicted urine nitrogen ($mg/kg^{0.75}/day$) for each harvest mouse. When I performed a regression of these new data on nitrogen intake ($mg/kg^{0.75}/day$), I found a value of EUN equal to 348.13 $mg/kg^{0.75}/day$ ($P < 0.001$; Fig. 38; Table 7, Equation 82). For modeling purposes, I also regressed estimated urine nitrogen ($mg/kg^{0.75}/day$) on % dietary nitrogen ($P < 0.001$; Fig. 39; Table 7, Equation 83).

This result still lies far above any reported value for EUN in rodents (Table 14); however, in light of the above discussion on the relationship between EUN, habitat, and dietary habits, I do not believe that this value is as unreasonable as it may seem. Harvest mice consume different food items (Kincaid and Cameron 1982a; Spencer and Cameron 1982; Sealander and Heidt 1990; Stancampiano and Caire 1995; Wilson and Ruff 1999) than those of the herbivorous rodents previously studied (Table 14). The typical diet of *R. fulvescens* is highly digestible by comparison, and contains foods of far higher protein content and quality. As such, this species may not make use of urea recycling mechanisms to sustain cellulolytic microbes and to recover nitrogen losses, at least not to the degree seen in herbivores (Hume et al. 1980; Campbell and MacArthur 1997; Hume 1999). Evidence also suggests that very small animals (< 300 g; McNab 1988), or those that consume readily digestible food items (McNab 1986; Elgar and Harvey 1987) such as seeds, tend to have higher metabolic rates than those predicted by Kleiber's (1961) equation. It is therefore reasonable to expect a correspondingly high level of EUN excretion (Smuts 1935).

Fig. 38.—Estimation of endogenous urinary nitrogen (EUN) for *R. fulvescens* by examining the relationship between estimated urinary nitrogen excretion ($mg/kg^{0.75}$/day) and average daily nitrogen intake ($mg/kg^{0.75}$) ($n = 53$; $Y = 348.13 + e^{0.001X}$; $P < 0.001$; $r^2 = 0.90$). EUN may be estimated from the intercept of this regression.

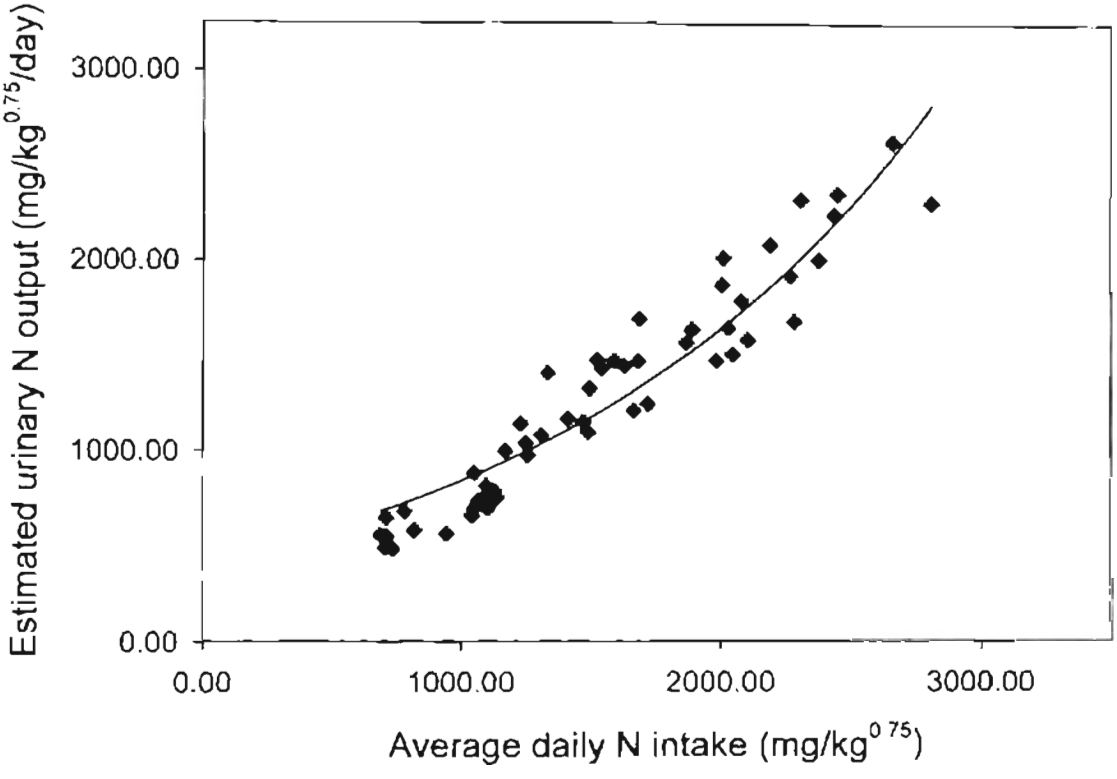
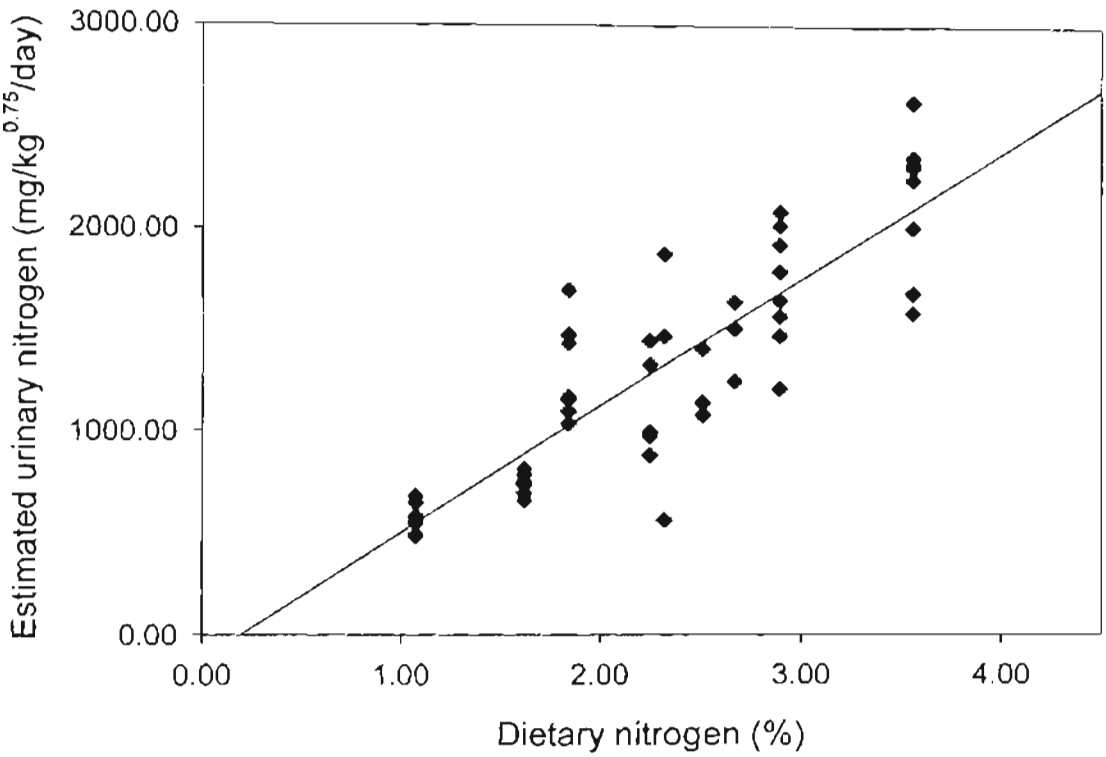

Estimated urinary N output (mg/kg^0.75/day)
3000.00
2000.00
1000.00
0.00
0.00
1000.00
2000.00
3000.00
Average daily N intake (mg/kg^0.75)

Fig. 39.—Relationship between estimated urinary nitrogen excretion ($mg/kg^{0.75}/day$) and nitrogen intake (%) for *R. fulvescens* ($n = 53$, $Y = -118.05 + 623.84X$, $P < 0.001$, $r^2 = 0.74$).


Estimated urinary nitrogen (mg/kg^0.75/day)
3000.00
2000.00
1000.00
0.00
0.00
1.00
2.00
3.00
4.00
Dietary nitrogen (%)

Digestive Efficiency

The relationships among DMD, AND, and dietary nitrogen in this study were as expected. Excessive amounts of certain nutrients, including protein, may impair digestion and assimilation of other nutrients (Pond et al. 1995). At the same time, nutrient deficiency often causes assimilation efficiencies to increase (Pond et al. 1995). These 2 forces combined to cause the observed negative relationship between DMD and nitrogen intake. Additionally, as dietary nitrogen decreases, MFN composes a larger portion of the measured fecal nitrogen. This increasing level of fecal nitrogen that is not of dietary origin causes apparent nitrogen digestibility to decrease (Robbins 1993; Pond et al. 1995); for this reason, many studies account for the presence of MFN by converting efficiencies of nitrogen assimilation to a truly digestible basis (Mould and Robbins 1981; Wallis and Hume 1992; Hume 1999).

Within-species differences in DMD and AND also may explain observed differences in nitrogen flux. Neither *S. hispidus* nor *P. leucopus* exhibited differences according to sex or origin in DMD or AND. However, female *R. fulvescens* could better process ingested feeds than males, potentially resulting in lower fecal nitrogen excretion and altered intake rates of digestible dry matter. Furthermore, differences according to sex for *R. fulvescens* were detected in the wet mass of large intestines (Appendix A). Although I suspect that the differences in the large intestine were an artifact of the necropsy procedure (the testes and associated reproductive organs made it difficult to clip the large intestine of males close to the anus), it is possible that alterations in intestine length or mass were responsible for the observed disparity in digestive efficiency.

True nitrogen digestibility (TND) is a measure of nitrogen absorption that corrects for the confounding influence of endogenous nitrogen excretion. Because MFN is not included in the estimation of total fecal nitrogen, TND strictly corresponds to the amount of exogenous (i.e., dietary) nitrogen that is broken down and absorbed across the intestinal mucosa (Robbins 1993; Pond et al. 1995). Values of TND measured in this study were lower those found in many other studies (Robbins 1993), which ranged from 83-100 %. However, Asleson et al. (1996) detected 77.5 % TND in white-tailed deer during antlerogenesis, comparable to the range of true digestibilities (76-82 %) detected in balance trials. They suggested that the nature of semisynthetic diets resulted in lower digestibility; that is, the pelleting process reduces feeds to small particles that pass rapidly through the digestive tract. Low retention time naturally results in reduced digestive efficiency (Robbins et al. 1993; Hilton et al. 1999). Likewise, the lower TND observed in my feeding trials may be attributed to the pelleted nature of experimental rations. Furthermore, small body size and a lack of insulation in metabolic cages may result in excessive heat loss (Aschoff 1981; Rübsamen et al. 1984; Bozinovic 1993; Phillips and Heath 1995) and higher intake rates, lowering the efficiency of digestion (Asleson et al. 1996).

Nitrogen Balance

As previously mentioned, I had difficulties reliably estimating nitrogen losses during the course of the balance trials, due to body-size limitations of the equipment used. A considerable degree of variability was introduced into my data as a result, which made it impossible to properly quantify dietary nitrogen requirements using the regression method for *S. hispidus* or *R. fulvescens*. Likewise, the relationship between

nitrogen balance and nitrogen intake for *P. leucopus* had a very low coefficient of determination and yielded a value for nitrogen requirements that was very high when compared with previously reported values for rodents (Table 1). In light of the degree of variability in the nitrogen balance data for all genera, I did not feel that this result was entirely reliable.

As a result, I used the factorial method (Karasov 1982; Robbins 1993) to extrapolate values for minimum nitrogen intake to maintain nitrogen balance. This method makes use of the principle that dietary requirements are determined by constant endogenous losses from the body of both fecal and urinary origin (all other losses, e.g., sloughing of skin, hair loss, respiratory losses, are assumed to be negligible; Robbins 1993). One should therefore be able to sum these losses, with a correction for nitrogen digestibility, to determine nitrogen requirements.

My calculations made use of 2 equations given by Karasov (1982) and Robbins (1993), respectively:

$$\text{N requirement (mg/kg/day)} = \text{EUN/BV} + [\text{MFN} * \text{DMI} * (1 - \text{BV})/\text{BV}]$$

$$\%\text{ dietary protein} = \{[\text{EUN} + \text{MFN (DMI)} * 6.25]/\text{DMI}/0.74] * 100$$

where BV = biological value of diet N (in cases where biological value is highly variable, as in the use of natural forages). Modification of these equations for the particulars of this study gives the following equation:

$$\text{Dietary N} = \{[(\text{EUN} * \text{BM}^{0.75}) + (\text{MFN} * \text{DMI} * 1000)]/\text{AND}\}/\ \text{BM}^{0.75}$$

where BM = average body mass (kg); dietary N is expressed in mg/kg$^{0.75}$/day , EUN in mg/kg$^{0.75}$/day, MFN in g/kg DMI/day, DMI in kg, and AND in percent. I used this equation to calculate requirements for each species, using measured or estimated values from balance trials for EUN, MFN, DMI, and AND. Estimated dietary nitrogen requirements were 217.2 mg/kg$^{0.75}$/day for *S. hispidus*, 528.8 mg/kg$^{0.75}$/day for *R. fulvescens*, and 349.8 mg/kg$^{0.75}$/day for *P. leucopus* These values lie within the range of reported dietary nitrogen requirements for rodents (Table 1). Although I still feel that the estimated requirement for harvest mice is somewhat high, I do not believe that it is entirely beyond reason, given the high digestibility and protein content of their natural diet (compared with herbivores).

Interspecific Comparisons

R. fulvescens had the highest DMI and DDMI of all 3 study species after correcting for metabolic body mass by scaling body mass to the 0.75 power. Therefore, they likely had the highest mass-specific metabolic rate. This phenomenon may be explained by 3 factors. First, as a consequence of their exceedingly small body size, harvest mice exhibit a large surface to body mass ratio and rapid rates of heat loss (Aschoff 1981; Rübsamen et al. 1984; Bozinovic 1993; Phillips and Heath 1995). High costs of thermoregulation and the aforementioned relationship between energy demands and feeding rate (Batzli and Cole 1979; Karasov 1982; Robbins 1993; Pond et al. 1995) cause an increase in voluntary intake. Second, McNab (1988) showed that as body mass decreases, rates of basal metabolism tend to be higher than those predicted by Kleiber

(1961) and Brody (1964). He suggested a scaling coefficient of 0.60 rather than 0.75 for species of body mass < 300 g. Finally, eutherians that consume highly digestible food items (e.g., vertebrates, herbs, nuts) typically have higher basal metabolic rates than species that eat poorly digested foods (e.g., invertebrates, leaves of woody plants; McNab 1986). As a result, *R. fulvescens* (a granivore) would be expected to have higher intake rates than *P. leucopus* (an omnivore).

In light of observed rates of feed intake and previous studies of dietary habits, there is a potential gradient according to dietary niche in the amount of nitrogen each species consumes per unit mass. Higher DMI and DDMI for *R. fulvescens* than *S. hispidus* or *P. leucopus*, coupled with the consumption of food items higher in nitrogen than those in the diet of cotton rats (Cameron and Spencer 1981; Kincaid and Cameron 1982; Spencer and Cameron 1982; Randolph et al. 1991), leads to much higher nitrogen intake by *R. fulvescens* than *S. hispidus*. *P. leucopus* show a more protein-dense diet than either *S. hispidus* or *R. fulvescens* (Lackey et al. 1985; Sealander and Heidt 1990; Wilson and Ruff 1999; McAdam and Millar 1999) and may at least partially compensate for the observed difference in intake rates between white-footed mice and harvest mice. Thus, I predict that the latter 2 species would show similar mass-specific rates of nitrogen intake under free-ranging conditions.

Excretion rates showed a similar disparity among these species. Although values of MFN did not differ statistically, rates of mass-specific fecal nitrogen excretion in response to nitrogen intake varied by species. The net effect was higher rates of mass-specific fecal nitrogen excretion for *R. fulvescens* over the range of dietary nitrogen in this study, followed by *P. leucopus* and then *S. hispidus*. Estimated values of MFN

followed this same trend (Table 7, Equations 1, 5, and 7). Urine excretion and estimates of EUN followed the same lines in terms of adaptation to dietary niche as fecal nitrogen, with loss of urinary nitrogen rank-ordered (from highest to lowest) as *R. fulvescens*, *P. leucopus*, and *S. hispidus*. In summary, *R. fulvescens* may be responsible for more deposition of nitrogen into the soil on the basis of metabolic body mass.

Estimates of digestive efficiency, nitrogen flux, and nitrogen requirements supported my hypothesis on the basis of dietary niche in part; that is, the herbivore (*S. hispidus*) showed lower mass-specific nitrogen flux rates, higher digestive efficiencies, and lower nitrogen requirements than a granivore and 2 omnivores. Findings on the 2 genera of mice were contrary to predictions. I hypothesized that selection pressures for nutrient assimilation and conservation would be lowest for *P. leucopus*, because this species routinely consumes higher levels of dietary nitrogen, and that a higher dietary nitrogen requirement for *P. leucopus* would ensue. However, digestive efficiencies are responsive to the breadth of the dietary niche. Generalists show higher rates of nutrient assimilation than specialists (Hilton et al. 1999); therefore the lack of selectivity shown by *P. leucopus* in the wild may be responsible for the observed higher digestibility of its diet as compared with *R. fulvescens*. A necessary consequence of reduced nutrient assimilation is a higher dietary need.

The particulars of the diet of *P. leucopus* also may predispose this species to higher digestive efficiencies. White-footed mice routinely consume large amounts of arthropods (Grant et al. 1977; Lackey et al. 1985; Sealander and Heidt 1990; Wilson and Ruff 1999; McAdam and Millar 1999), which contain large amounts of indigestible protein in the form of chitin in their exoskeletons. Such a diet demands lower basal

metabolic rates (McNab 1986) and therefore less daily DMI, higher retention times, and more thorough digestion (Robbins et al. 1993; Hilton et al. 1999). Studies on the gastrointestinal physiology of raptors (Akaki and Duke 1999; Hilton et al. 1999) supports the assertion that largely insectivorous species demonstrate high efficiencies of nutrient assimilation.

Interspecific differences in nutritional requirements have been attributed to habitat variation, with animals living in unpredictable environments tending to have a lower need for dietary nitrogen (Wallis and Hume 1992). *Peromyscus* spp. exhibit such environmental variation; *P. maniculatus* is widely distributed in habitat and geography, and *P. leucopus* inhabits edge habitats between wooded and grassland areas. The range of both peromyscines, unlike those of the other 2 species, also is centered in the United States; animals living in such northern latitudes would experience more seasonality. The net result of this high degree of environmental variation may be the lower need for dietary nitrogen observed in my study. This line of reasoning, in addition to my predictions based on dietary niche, also may partially explain the lower nitrogen requirements of *S. hispidus*, given the stochastic nutritional environment of this species (Lochmiller et al. 2000). *R. fulvescens*, on the other hand, maintains a fairly constant level of high-quality items in its diet through seasonal shifts in resource utilization (Kincaid and Cameron 1982; Spencer and Cameron 1982). Thus, this species avoids drastic variations in food availability, even though it shares the same habitat as *S. hispidus*.

Differences in overall digestive efficiency may be attributed either to differences in the length or mass of gastrointestinal organs (such that some individuals possess more

cells with which to carry out digestive and absorptive processes), or to differences in the efficiency of digestive enzymes or gut microflora (Corp et al. 1997; Hilton et al. 1999; McWilliams et al. 1999). Hilton et al. (1999) found an association among shorter, less massive gastrointestinal tracts, rapid passage rate, and lower assimilation efficiency in raptors. However, Corp et al. (1997) found no difference in absorption efficiency between 2 populations of wood mice with different digestive tract lengths as a result of contrasting resource availability.

I believe that the observed differences in assimilation efficiency among the 3 genera studied may be attributed to shifts in digestive function rather than gastrointestinal morphometry. With only 1 exception, I found no differences between subadult *S. hispidus* and *R. fulvescens* in the length or mass of any digestive organ (Appendix A; similar data from *P. leucopus* are not available). Alternative physiological explanations for higher digestive efficiency in *S. hispidus* include: 1) gastrointestinal tissues that secrete more digestive enzymes per cell (i.e., more active secretory cells); 2) digestive isozymes with higher rates of enzymatic action or higher affinity for their designated substrate (i.e., higher enzymatic activity; Corp et al. 1997; McWilliams et al. 1999); 3) proteins within the intestinal mucosa that transport digested particles from the lumen to the portal system more rapidly (i.e., more complete absorption; Karasov et al. 1996; Corp et al. 1997; McWilliams et al. 1999); or 4) a substantively different community of bacteria and protozoa (especially in the cecum) that breaks down foodstuffs (e.g. cell wall components) more completely (Blossman-Myer and Stangl 1999).

Reproductive Requirements

General

Reproducing cotton rats and harvest mice showed consistent rates of DMI across all dietary nitrogen levels, indicating a lack of compensatory intake on low-nitrogen diets. Results also indicated that treatments were assigned independently of the number of days before parturition that females were first offered experimental rations. As planned, the average length of time before parturition that females began feeding trials also corresponded to a point two-thirds of the way through the gestation period for each species. Although I found a difference by origin in maternal body mass for cotton rats, the randomness of treatment assignments, as well as the correction of all data for metabolic body mass, should diminish origin effects on experimental observations. The lack of a difference in litter size between captive-born and wild-caught females corroborates this supposition.

Breakpoint Analysis

There are no published data on the reproductive requirements of *R. fulvescens*; therefore, I could not compare my findings to those of other studies. The estimated dietary nitrogen requirement for reproduction in *S. hispidus*, as calculated from breakpoint models, was somewhat higher than those previously reported (1.2 %, Randolph et a. 1995; 1.7-1.9 %, Hellgren and Lochmiller 1997; Table 2). However, these studies based reproductive requirements on the total amount of protein contained in the bodies of 5 pups at 12 days postpartum. I used litter growth rates at 12 days after birth. Use of optimal growth to predict dietary requirements was developed for domestic animals in production settings, and may not be entirely relevant to free-ranging species.

Under natural conditions, few females are able to obtain sufficient resources for their offspring to grow to their genetic potential.

Previous investigations on lactational responses to changing maternal nutrition have shown that the composition of milk does not change under dietary restriction (Lochmiller 1984; Glem-Hansen 1979). Rather, milk production decreases after maternal nutrient intake drops below a certain critical point (Glem-Hansen 1979; Robbins 1993). Thus, the observed decline in offspring growth rates below each breakpoint may be attributed to substantive changes in milk quantity rather than composition, and changes in growth rate are likely not obscured by alterations in nursling body composition.

Reproductive and Survival Responses to Maternal Nitrogen Intake

I found positive responses of litter size, litter mass, and individual pup mass to maternal nitrogen intake throughout the lactation period in *S. hispidus*. These data support the findings of Cameron and McClure (1988) that cotton rats are capable of wide variation in many aspects of their life history, and Derting's (1997) assertion that intraspecific variations in productivity, body size, and rates of gain are determined by nutrient availability, including that of protein. Lochmiller et al. (2000) altered dietary levels of casein among postweanling cotton rats and found changes in growth, body mass, and efficiency of gain as a result. My findings extend this work by documenting an equal level of variation in neonatal patterns of growth and development under changing nutritional conditions.

Interspecific Comparisons

Interspecific differences in DMI for reproducing females reflect my findings from maintenance feeding trials. Lower efficiencies of nutrient assimilation for *R. fulvescens*,

along with potentially higher rates of energy metabolism, necessitate higher feed intake per unit body mass to meet physiological needs. Because their life history dictates larger young relative to maternal body mass, harvest mice also put relatively more resources into each litter. Small body size also hinders the ability of female *R. fulvescens* to build sufficient reserves to meet demands of lactation (Oftedal 2000); therefore, they must consume higher levels of dietary nutrients.

Results of breakpoint regressions were contrary to predictions on the basis of dietary niche, with a surprising finding of a higher dietary nitrogen requirement for *S. hispidus* (a generalist herbivore) than for *R. fulvescens* (a granivore). However, my analysis reveals distinctive patterns in reproductive investment that point to a strong influence of life-history strategy on the allocation of nutritional resources. These species represent 2 opposite approaches to life-history strategy. *R. fulvescens* has highly seasonal and bimodal reproductive patterns, with relatively small litters (2-5) of altricial young (Table 5) typical of more density-dependent or *K*-selected species (Boyce 1988; Roff 1992; Stearns 1992; Pianka 2000). This species also is longer lived than cotton rats (Cameron 1977; Spencer and Cameron 1982; Wilson and Ruff 1999). *S. hispidus* is typically short-lived, can reproduce at any time given adequate food availability, reproduces fairly often, and produces large litters (6-9) of precocial young (Table 5; Cameron and Spencer 1981; Sealander and Heidt 1990; Wilson and Ruff 1999). This pattern is representative of *r*-selected or density-independent species (Boyce 1988; Roff 1992; Stearns 1992; Pianka 2000).

S. hispidus also show a large degree of intraspecific variation in life-history traits within and among wild populations in different geographic regions (Doonan and Slade

1995; Cameron and McClure 1988; Derting 1997). Cameron and McClure (1988) concluded that, although the latitudinal patterns in litter size among populations had a genetic basis, phenotypic plasticity seen within populations showed low heritability and could be attributed to differences in rates of ovulation, fertilization, implantation, and embryo survival. Derting (1997) also noted that subspecific variation in life-history traits (e.g., productivity, body size, growth rates, litter size) was a consequence of environmental factors rather than genetic hardwiring. All of these factors are potentially affected by diet (Batzli 1985; White 1993; Eshelman and Cameron 1996; Lochmiller et al. 2000).

The capacity of cotton rats to display variation in reproductive traits explains observed changes in reproductive effort by females of this species as dietary nitrogen decreases. All measurements of nursling cotton rat development responded to maternal nitrogen intake In contrast, the only significant relationships for harvest mice were for individual pup mass at birth and weaning. For this last variable, a greater proportion of the variation in individual mass was explained by maternal diet for cotton rats (44%) than for harvest mice (21%).

I believe that changes in the litter size of cotton rats may be attributed to the influences of maternal diet on rates of embryo survival. During the course of my research, I noted the birth of several stillborn rats—although I could not analyze the effect of diet on embryo mortality because mothers quickly cannibalized all stillborns after parturition. However, given the significant relationship between litter size and maternal diet, I suspect that females on lower-protein rations had a higher incidence of fetal mortality (Cameron and McClure 1988).

Differential neonatal survival in cotton rats and harvest mice may be explained by dietary niche considerations (because granivores require more dietary nutrients to sustain reproduction), life-history strategy (because bet-hedging species are more likely to abandon current reproduction in favor of future resource availability), or allometric effects on maternal nutrient stores and mobilization (Oftedal 2000). The patterns in offspring survival for *S. hispidus* and *R. fulvescens* in this study were striking. Below 1.5 % dietary nitrogen, most nursling harvest mice were either killed and cannibalized by their mothers, or were too small and weak at weaning to survive in the absence of maternal care. By contrast, all juvenile cotton rats in this study survived until weaning and beyond, regardless of the level of maternal dietary nitrogen. Similar patterns were found by Sikes (1995b, 1996b) with food-restricted northern grasshopper mice (*Onychomys leucogaster*) and eastern woodrats (*Neotoma floridana*). Grasshopper mice cannibalized their young after reaching a certain amount of maternal mass loss, whereas woodrats tried to raise their pups under all conditions. Dead nursling woodrats were found only as a result of malnutrition, rather than maternal abandonment. These species exhibit life-history traits that are *K*-selected relative to other rodents (R. S. Sikes, pers. comm.). Therefore, neonatal woodrats probably experienced higher survival because female woodrats (which are much larger than grasshopper mice) were able to tap into more nutrient stores for gestation and lactation (Oftedal 2000; R. S. Sikes, pers. comm.). However, because body size, dietary niche, and life history confound each other in this study, I am uncertain which effect best explains my findings.

I compared nitrogen requirements for offspring survival and optimal growth for each species (Table 15), using a survival rate of 50% to generate a nitrogen requirement

Table 15.—Dietary nitrogen requirements for survival and maximal growth in nursling *S. hispidus* and *R. fulvescens*. Standard errors are shown in parentheses.

Species	Requirement (%)	
	Survival	Maximal growth
S. hispidus	< 1.08	2.31 (0.16)
R. fulvescens	1.34 (0.08)[a]	1.29 (0.10)[a]

[a]Values not significantly different ($Z = 0.39$, $P = 0.70$).

for survival in juvenile harvest mice. These data uphold the view that reproduction and growth in cotton rats are more plastic with regard to nitrogen availability than harvest mice. Nursling *S. hispidus* can survive at maternal intakes of 1.08 % dietary nitrogen, although growth does not become optimal until 2.31 % nitrogen. On the other hand, juvenile *R. fulvescens* survive and grow maximally within a narrow range (1.3 – 1.7 % dietary nitrogen). These data support my prediction that granivores require more nitrogen than herbivores to reproduce successfully. They also underscore the physiological flexibility displayed by cotton rats, as predicted by the literature (Cameron and McClure 1988; Derting 1997; Lochmiller et al. 2000).

My data on maternal mass loss during lactation may explain the ability of juvenile cotton rats to survive at all levels of dietary nitrogen presented in this study. Female cotton rats sacrificed body reserves to produce sufficient milk to keep their offspring alive, reflected in the larger amount of mass lost by mother on low-nitrogen diets. Female harvest mice were less able to mobilize maternal resources in this way, as there was no such decrease in body mass for malnourished mothers. This species may simply lack the body stores that cotton rats seem to possess, just as Sikes (1995b, 1996b, pers. comm.) observed in grasshopper mice. Smaller mammals are often unable to convert nutrient surplus into excess body mass because of the relationship among body size, energy expenditure, and gastrointestinal capacity (Oftedal 2000). Furthermore, offspring of small mammals have higher mass-specific energy demands, making it unlikely that maternal reserves may provide an adequate supply of nutrients to the production of milk (Oftedal 2000). Thus, *R. fulvescens* cannot compensate for an inadequate diet with a loss in maternal mass.

I conclude that the differential species response in reproduction to varied dietary nitrogen is tied to life-history strategy and constrained by body mass considerations described above. Life-history theory predicts that species native to unpredictable environments (including variable nutritional availability) should demonstrate phenotypic flexibility in reproductive traits (Stearns 1992; Lochmiller et al. 2000). Thus, individuals with a low chance of survival to the next reproductive bout, such as *S. hispidus*, should reproduce under any conditions without placing too many demands on maternal nutrient reserves. Cotton rats rely on high reproductive output, phenotypic plasticity in litter size and offspring growth rates, and mobilization of limited maternal stores to improve fitness. Reproduction can occur at very low levels of nitrogen intake, but the species retains the flexibility to increase maternal investment with increasing nitrogen.

Species with a high probability of future survival, on the other hand, should forego current reproduction if female survival and future fitness of offspring are compromised. By minimizing maternal mortality, lifetime fitness is optimized (Stearns 1992; Lochmiller et al. 2000). Because harvest mice live longer but reproduce less often and in smaller numbers, they adopt this bet-hedging strategy for reproductive investment. Nursling growth and survival analyses showed that maximum growth rate is sustained as long as possible as dietary nitrogen decreases. Once growth rate is compromised by decreasing nitrogen intake, offspring survival deteriorates rapidly. Harvest mice can afford this all-or-nothing strategy for an individual reproductive bout because they may have a higher probability of surviving to the next reproductive bout than cotton rats. For harvest mice, intensive investment in a small litter also maximizes postweaning survival of young and individual fitness.

Although I was unable to obtain sufficient sample size from either species of *Peromyscus* to compare their reproductive responses to nitrogen availability, I observed certain trends among the few litters that were produced. As with harvest mice, I did not detect any changes in neonatal litter size in response to maternal nitrogen. I also noticed that the female on the lowest level of dietary nitrogen was quick to cannibalize her offspring in response to dietary restriction; young of this litter did not survive beyond 4 days of age, much younger than any of the cannibalized harvest mice. These limited observations support my prediction that *P. leucopus* require at least as much dietary nitrogen as *R. fulvescens*. Because *P. leucopus* and *R. fulvescens* show similar life-history strategies but different dietary habits, and because *P. leucopus* and *P. maniculatus* share similar diets but have somewhat different reproductive patterns, further research on this genus would prove interesting, in terms of intra- and intergeneric comparisons.

Postweaning Growth

General/Estimation of Requirements

Changes in dietary protein affect the development of juvenile rodents. Protein availability constrains growth and affects age at maturity in free-ranging deer mice and cotton rats (Cameron and Eshelman 1996; McAdam and Millar 1999). Derting (1997) showed that high-protein diets fed to juvenile cotton rats under laboratory conditions caused observed subspecific variation in field growth rates to disappear. She concluded that developmental responses to protein intake are subject to environmental rather than genetic influences.

Generally, I found no relationship between DMI ($g/kg^{0.75}$/day) and dietary nitrogen (%), weanling sex, or maternal origin, although there was a trend toward

compensatory intake and a maternal origin effect in cotton rats at 1 week after weaning. The observed decline in DMI over time for both species (with the exception of week 2 DMI for *R. fulvescens*) is a reflection of typical patterns of growth for mammalian species. Most weanlings grow rapidly immediately after weaning, with a subsequent decrease in growth rates as the animal ages and nears its adult body mass (Zullinger et al. 1984). Therefore, the need for nutrient uptake decreases, and feed consumption drops off over time (Pond et al. 1995).

Only 2 data sets for postweaning growth rates yielded significant breakpoint models that I could use to quantify requirements for postweaning gain. The most meaningful of these, i.e., the regression with the least amount of variation and from the most significant time frame, was my analysis of daily growth rates of cotton rats during the first week after weaning. Because the period of most rapid gain occurs just after weaning (Zullinger et al. 1984), minimum requirements for postweaning growth are best determined at this time.

The estimate for minimum dietary requirement for maximum growth at week 1 postweaning (1.96 % nitrogen or 12.3 % crude protein) was consistent with previous work. Lochmiller et al. (2000) estimated minimal requirements at 12% crude protein, with 16% required for optimal growth. Cameron and Eshelman (1996) found greatly improved growth rates of juveniles fed an 11% crude protein dicot diet as opposed to those on a 4% monocot diet. The magnitude of the calculated dietary nitrogen requirements for growth (1.62 g/kg$^{0.75}$/day at week 1 and 1.49 g/kg$^{0.75}$/day at week 2), as compared to reproductive (2.92 g/kg$^{0.75}$/day) and maintenance (0.22 g/kg$^{0.75}$/day) requirements, was also consistent with studies showing postweaning growth requirements

to be the same order of magnitude as for gestation and lactation (with nitrogen requirements for maintenance dramatically lower; Robbins 1993).

Maternal/Individual Effects on Growth

Many multiple regression analyses showed a lack of relationship between growth parameters and any potential independent variable (Table 12). I speculate that the regression of body mass at week 6 postweaning was not significant for *R. fulvescens* because harvest mice had already reached their adult body mass at that point in time. Average time to adult mass in this species was 47.5 days of age (26.5 days postweaning), and some individuals reached their adult mass at 30-40 days of age (10-20 days postweaning). Therefore, any differences in juvenile body mass due to nutritional influences would be masked soon after this point in time.

Maternal and postweaning nitrogen intake had a positive influence on measurements of body mass (mass at weeks 3 and 6, estimated adult mass) and growth rates (e.g., mass gain during the first 3 weeks after weaning) for *S. hispidus* (Table 11). These findings are consistent with previously reported findings on results of dietary protein restriction in cotton rats (Cameron and Eshelman 1996; Lochmiller et al. 2000) and deer mice (McAdam and Millar 1999). Sikes (1996a) failed to find a relationship between maternal food restriction and adult mass of offspring in *O. leucogaster* (although he did find a positive influence of maternal diet on weaning mass); however, experimental treatments were initiated after parturition, and the effects of prenatal malnutrition (as in the current study) were not investigated.

In keeping with earlier findings (Cameron and Eshelman 1996; McAdam and Millar 1999), my research showed a generally negative relationship between nitrogen

intake and time to adult body mass (Table 11). Derting (1997) failed to find an effect of environmental factors on time to sexual maturity for *S. hispidus*; however, given the decoupling of first estrus and adult body mass in this species (Cameron and Spencer 1981; Wilson and Ruff 1999), the influence of nutrition on these 2 variables may differ. Furthermore, average adult mass and time to adult mass as calculated from juvenile growth curves are in keeping with published life-history data for *S. hispidus* and *R. fulvescens* (Table 5; Cameron and Spencer 1981; Spencer and Cameron 1982; Sealander and Heidt 1990; Nowak 1997; Wilson and Ruff 1999).

All measurements of nitrogen conversion efficiency were negatively affected by nitrogen intake (Table 11). Thus, individuals on poor planes of nutrition achieved more gain in mass on a lower nitrogen intake or, conversely, animals with high nitrogen intake displayed lower absorption efficiency as digestive enzymes and intestinal transport proteins became saturated (Pond et al. 1995). The decrease in mean efficiency of nitrogen utilization during the first 3 weeks after weaning may be attributed to the same cause as the observed decrease in DMI over time; that is, the majority of juvenile growth occurs just after weaning and younger individuals have a greater need for efficient gain. I also found that effects of postweaning diet on nitrogen conversion were stronger than those of maternal diet, likely because current nitrogen intake is of highest importance to such rapidly-growing animals. Higher levels of DMI observed in juveniles during the first week after weaning, along with their higher rates of nutrient utilization, mean that younger animals have a tremendous capacity to increase their body mass.

Interspecific Comparisons

My comparison of species means for DMI showed results similar to those obtained in maintenance and reproductive trials. In all cases, *R. fulvescens* showed higher intake rates, undoubtedly due to lower digestive efficiencies (as shown by balance trial data) and perhaps higher mass-specific metabolic rates. Efficiencies of nitrogen utilization were much higher in cotton rats, because the restrictions of an herbivorous diet and subsequent higher digestive efficiencies result in greater assimilation of dietary nitrogen (Batzli and Cole 1979; Robbins 1993; Hume 1999). Thus, this species can achieve higher rates of growth with the same nitrogen intake. Male *S. hispidus* also showed higher conversion efficiencies than females. This result was not surprising, given the degree of sexual dimorphism in this species (Cameron and Spencer 1981; pers. obs.) and the positive effect of testosterone on feed conversion (Jenkins et al. 1988; DeHaan et al. 1990). Males grow faster to reach higher body masses (Cameron and Eshelman 1996; this study) and have a need for more economical gain. The degree of dimorphism in *R. fulvescens* is not as apparent (Spencer and Cameron 1982), therefore no sex differences in nitrogen utilization efficiency were detected.

Analysis of growth curve parameters pointed to another contrast between cotton rats and harvest mice. The maximum growth rate for *R. fulvescens* was higher than measured growth rates at any point postweaning (Table 10). Maximum growth rates for *S. hispidus*, as estimated from growth curves, was less than the mean growth rate at week 1 postweaning. I infer from these data that maximum growth of harvest mice occurred before weaning, whereas cotton rats reached their highest growth rates within the first 2 weeks after weaning. Such a conclusion would be consistent with patterns of growth in

altricial and precocial young. Because altricial neonates cannot regulate their own body temperature, relying instead on body heat from their mother, they incur reduced energetic costs of thermoregulation and can channel dietary energy into growth (McClure and Randolph 1980; Waldschmidt and Müller 1988; Webb and McClure 1989). As a result, these species experience a period of rapid growth and development soon after birth that slows shortly after weaning (McClure and Randolph 1980; Waldschmidt and Müller 1988). Precocial offspring develop homeothermy relatively quickly and must utilize dietary energy for thermoregulation as well as growth, resulting in linear rather than multiphasic neonatal growth (McClure and Randolph 1980; Waldschmidt and Müller 1988).

Many invertebrate and vertebrate organisms have a genetic capacity for developmental plasticity in response to resource availability (Stearns and Koella 1986), including *S. hispidus* (Cameron and McClure 1988; Lochmiller et al. 2000), and especially the subspecies that occurs most commonly in the southern Plains (Caire et al. 1989; Derting 1997). This ability to display phenotypic variation was evident in my study, because cotton rats demonstrated strong relationships between dietary nitrogen and almost all growth parameters. *S. hispidus* also experienced a greater influence of maternal diet than postweaning diet on postweaning growth and development. This may be attributed to the highly variable maternal environment experienced by nursling cotton rats. Because maternal resource investment changed with increasing dietary nitrogen, weanling rats displayed a wide variety of body sizes and developmental trajectories. Although compensation for maternal shortcomings (i.e., postweaning compensatory

growth) is possible for certain species under certain conditions (Sikes 1998), cotton rats in my study apparently did not have this ability.

Harvest mice exhibited less variation in juvenile development, because a number of regressions yielded no relationship (e.g., adult and 6-week mass, all measurements of growth rate) or weak relationships (e.g., time to adult mass) between measures of neonatal development and diet. Growth rate and body size of *R. fulvescens* were fairly inflexible to variable dietary nitrogen, as I observed during reproductive feeding trials. Nutrient-restricted female harvest mice showed a lack of plasticity in reproductive investment during the pre-weaning period. Those that were subject to excessive nitrogen limitation simply terminated reproduction; hence, any weanlings that would have been potentially constrained by maternal environment were already dead. Surviving offspring did not show much variation in pre-weaning development as dietary nitrogen changed; therefore most weanlings shared a common maternal background. The logical consequences of such inflexibility in juvenile growth patterns would be delayed sexual maturity among malnourished individuals, because nutritional resources are directed toward growth rather than reproductive maturation (Lochmiller et al. 2000). However, such longer-lived species also have the luxury of time, and future reproduction (and therefore lifetime fitness) will likely have a positive relationship with larger body size (Stearns 1992; Lochmiller et al. 2000).

Many studies on nutrient restriction in young, growing animals have shown that individuals sufficiently constrained to retard normal rates of growth and development will experience a corresponding increase in growth rate upon removal of nutritional deprivation (Wilson and Osbourn 1960; Sikes 1996a; Lepczyk et al. 1998; Sikes 1998;

Lochmiller et al. 2000). This acceleration in rates of gain during nutritional rehabilitation is accomplished through gastrointestinal modification to allow greater intake rates, higher efficiency of digestion and assimilation, and more efficient feed conversion (Watkins et al. 1991; Sikes 1996a; Lepczyk et al. 1998; Sikes 1998). This phenomenon is commonly called compensatory growth (Wilson and Osbourn 1960; Sikes 1996a, 1998; Lochmiller et al. 2000).

I defined compensatory growth during these feeding trials as increased mass gain during the first 3 weeks postweaning for offspring of mothers on lower-protein diets. According to this definition, I failed to find evidence for compensatory growth in juvenile cotton rats or harvest mice. Although there were higher efficiencies of nitrogen conversion during the first week postweaning for the young of lower-nitrogen mothers, these efficiencies did not translate into differences in absolute growth rates or body mass. Sikes (1998) and Lochmiller et al. (2000) hypothesized that species found in nutritionally unpredictable environments (such as *S. hispidus*; Schetter et al. 1998) would preferentially use their energy reserves for early maturation and reproduction, limiting resources available for compensatory gain. Therefore, one would not expect cotton rats to compensate for inadequate maternal nutrition in this way. However, Lochmiller et al. (2000) also suggested that maternal nutrient reserves serve to "buffer" their young from a nutritionally inadequate environment. I found evidence for this in reproductive trials, as female rats on low-nitrogen diets experienced greater rates of mass loss. Consequently, pre-weaning planes of nutrition may not have been as restrictive as they appeared.

Free-ranging harvest mice, on the other hand, have sufficient resource availability to achieve compensatory gain and adequate time before sexual maturity to counteract

maternal shortcomings. However, this species exercises bet-hedging to an extreme at the nursling stage, aborting current reproductive bouts in hopes of future nutritional availability. Individuals that show low rates of gain before weaning as a result of maternal malnutrition are also those that are most likely to be cannibalized, as they are too much of a drain on their mothers' own resources. As a result, I did not detect any difference in 3-week gain among surviving juveniles. It seems that the type of animal ideally suited to exhibit compensatory gain is one whose life-history strategy lies somewhere between those of *S. hispidus* and *R. fulvescens*. I predict that, had I been able to conduct growth trials with either species of *Peromyscus* (especially *P. maniculatus*), I would have found evidence of such a postweaning "catch-up," as the life histories of these 2 species are more moderate. Compensatory growth has been demonstrated in food-restricted northern grasshopper mice (*Onychomys leucogaster*) and eastern woodrats (*Neotoma floridana*), which demonstrate considerable parental investment (*O. leucogaster*), or a significant delay in maturation (*N. floridana*) (Sikes 1996a, 1998).

Ecological Implications

My results provide empirical data necessary to model the quantitative role of small mammals in nitrogen cycling in this region, given effects of body mass, dietary niche, and biomass. Although harvest mice and white-footed mice showed higher nitrogen flux rates on a per-unit-mass basis, cotton rats are much larger and have higher net nitrogen flux per individual. On the other hand, the natural diets of white-footed mice are likely higher in nitrogen than those of harvest mice, which are in turn higher than those of cotton rats (Kincaid and Cameron 1982, Lackey et al. 1985, Randolph et al. 1991, Eshelman and Cameron 1996, McAdam and Millar 1999). When dietary niche

partitioning among these species is taken into account, interspecific differences in flux may diminish. Populations of *S. hispidus* are highly cyclical in nature, with extreme variations in population density, depending on food availability (Cameron 1977; Sealander and Heidt 1990; Wilson and Ruff 1999). *R. fulvescens* shows a certain degree of population cyclicity, though to a lesser extent than cotton rats (Cameron 1977; Cameron and Kincaid 1982). Populations of *P. leucopus* are typically more stable (Lackey et al. 1985; Wilson and Ruff 1999). Species composition shows a corresponding cyclical pattern, with dominance trading off among the 3 genera (J. L. Parsons, pers. obs.). As a result, the overall contribution of small mammals to nitrogen cycling may increase or decrease, depending on which species is more abundant at any particular time.

Rates of nitrogen influx and efflux observed during maintenance feeding trials allow estimation of these rates in the field. A series of sample calculations, using observations of mean body mass and feed intake, reported values of forage nitrogen content, and equations from Table 7 can examine the degree to which each species contributes to environmental nitrogen flux (Table 16). These calculations yield net rates of daily nitrogen flux of 217 mg for an average individual cotton rat, 119 mg for a harvest mouse, and 128 mg for a white-footed mouse. Thus, a single cotton rat is responsible for almost twice the nitrogen flux of a single harvest mouse or white-footed mouse when consuming natural forages. In effect, 1 cotton rat equals 2 mice in terms of nitrogen turnover.

In a similar fashion, I used data on average DMI for each species during reproductive feeding trials (Table 8) to calculate whole-body rates of nitrogen intake by lactating females (Table 17) and postweaning juveniles (Table 18) in the wild. My

Table 16.—Estimated nitrogen flux by free-ranging adult *S. hispidus*, *R. fulvescens*, *P. leucopus*, and *P. maniculatus* at maintenance.

Species	Rate of intake or excretion/kg$^{0.75}$/day			Whole-body rate of daily intake or excretion			
	g DMI[d]	mg fecal N[e]	mg urinary N[f]	g DMI	mg dietary N	mg fecal N	mg urinary N
S. hispidus[a]	48.24	191.34	227.45	11.63	116.27	46.12	54.82
R. fulvescens[b]	68.46	465.52	1261.12	2.64	52.71	17.92	48.55
Peromyscus spp.[c]	50.77	387.41	751.59	2.70	67.50	20.60	39.97

[a]Assumed body mass of 150 g and 1 % dietary nitrogen (Cameron and Spencer 1981; Randolph et al. 1991; Cameron and Eshelman 1996; Eshelman and Cameron 1996).

[b]Assumed body mass of 13 g and 2 % dietary nitrogen (Kincaid and Cameron 1982; Spencer and Cameron 1982; Stancampiano and Caire 1995).

[c]Assumed body mass of 20 g and 2.5 % dietary nitrogen (McAdam and Millar 1999; Lewis et al. 2001).

[d]From mean dry matter intake measured during balance trials.

[e]Calculated from Equations 3, 6, and 9 (Table 7).

[f]Calculated from Equations 4, 80, and 10 (Table 7).

Table 17.—Estimated nitrogen intake by lactating *S. hispidus* and *R. fulvescens* under free-ranging conditions.

Species	Daily whole-body intake at given stage of lactation									
	Day 5		Day 10		Day 15		Day 20		Peak[d]	
	DMI (g)[c]	N (mg)	DMI (g)[c]	N (mg)	DMI (g)[c]	N (mg)	DMI (g)[c]	N (mg)	DMI (g)[c]	N (mg)
S. hispidus[a]	18.00	315.08	26.06	456.05	39.50	691.29	____	____	30.48	533.32
R. fulvescens[b]	4.91	122.78	5.32	132.99	6.43	160.65	7.75	193.65	6.21	155.16

[a]Assumed body mass of 150 g and 1.75 % dietary nitrogen (Cameron and Spencer 1981; Cameron and Eshelman 1996).

[b]Assumed body mass of 14 g and 2.5 % dietary nitrogen (Kincaid and Cameron 1982; Spencer and Cameron 1982; Stancampiano and Caire 1995).

[c]Calculated from mean dry matter intake measured during feeding trials (see Table 8).

[d]Peak lactation = 12 days postpartum for *S. hispidus* and 14 days postpartum for *R. fulvescens*.

Table 18.—Estimated nitrogen intake by juvenile *S. hispidus* and *R. fulvescens* under free-ranging conditions.

Species	Daily whole-body intake at given point postweaning					
	Week 1[c]		Week 2[d]		Week 3[e]	
	DMI (g)[f]	N (mg)	DMI (g)[f]	N (mg)	DMI (g)[f]	N (mg)
S. hispidus[a]	8.07	141.18	9.28	162.43	9.40	164.52
R. fulvescens[b]	3.54	88.57	4.11	102.77	4.10	102.52

[a]Assumed 1.75 % dietary nitrogen (Cameron and Eshelman 1996).

[b]Assumed 2.5 % dietary nitrogen (Kincaid and Cameron 1982; Spencer and Cameron 1982; Stancampiano and Caire 1995).

[c]Assumed body mass of 45 g for *S. hispidus* and 8.5 g for *R. fulvescens*.

[d]Assumed body mass of 60 g for *S. hispidus* and 10 g for *R. fulvescens*.

[e]Assumed body mass of 70 g for *S. hispidus* and 11 g for *R. fulvescens*.

[f]Calculated from mean dry matter intake measured during feeding trials (see Table 10).

calculations for intake during lactation assumed no significant change in body mass from parturition to weaning, although data on *S. hispidus* provide some evidence for loss of maternal body mass. Therefore, intake rates may be somewhat lower for this species toward the end of the lactation period. Nevertheless, the potential difference in environmental impact between these 2 species may still be appreciated. On natural diets, female cotton rats consume 157 % more nitrogen at the beginning of the lactation period, 244 % more at peak lactation, and 257 % more near weaning than reproducing harvest mice. Juvenile cotton rats ingest approximately 60 % more nitrogen than growing harvest mice, despite the difference in forage quality. The increase in intake rates by reproducing females also underscores the extreme nutritional demands of late lactation (Robbins 1993).

Implications of my results for the effects of nitrogen supplementation on the structure of rodent assemblages in the tallgrass prairie are powerful. Nitrogen availability may have strong influences on the population dynamics of small mammals, especially cotton rats. Cameron and McClure (1988) postulated that geographic disparities in dietary protein could explain observed differences in small-mammal growth and reproduction in southern latitudes. They also found increased litter size in *S. hispidus* with greater variation in temperature and primary productivity. Despite considerable subspecific variation in life-history traits, geographically separated populations of cotton rats showed similar digestive capabilities and were equally responsive to high-quality diets (Derting 1997). These results suggest that these populations were chiefly constrained by nutrient availability.

Nitrification of the soil could improve reproduction by making available more high-quality habitat for females, both in terms of cover and biomass of high-protein forages. Food supplementation in free-ranging cotton rat populations led to increased reproduction and immigration to supplemented patches, resulting in increased population densities (Doonan and Slade 1995). Eshelman and Cameron (1996) and Randolph and Cameron (2001) documented movements of pregnant females from patches dominated by grasses and sedges (4% crude protein) to mixed patches with higher concentrations of dicots (11% crude protein). Thus, cotton rats may temporarily shift their habitat to better meet nutritional needs. Nitrogen supplementation also could decrease seasonal variation in productivity, enabling demographic increases in regions or at times of year when populations are normally limited by nitrogen availability. For example, Cameron and Eshelman (1996) found that the monocots in typical habitats of cotton rats in southeastern coastal Texas contained only 4% crude protein, well below those necessary for growth and reproduction (Randolph et al. 1995; Eshelman and Cameron 1996; this study). Supplementation of experimental plots with protein induced population redistribution and changes in demography (Eshelman and Cameron 1996) similar to those seen by Doonan and Slade (1995).

Phenotypic plasticity in reproductive traits may allow *S. hispidus* to take advantage of higher nutrient availability with a corresponding shift in maternal investment during gestation. Cotton rats experienced an improvement in virtually every measured characteristic of reproduction and growth as a result of increased dietary nitrogen availability. Evidence suggests that the same phenomenon may occur among species native to other habitats as well. Grant et al. (1977) reported higher pregnancy

rates among prairie voles (*Microtus ochrogaster*) in shortgrass prairie supplemented with nitrogen and water than on plots treated with irrigation only. Prairie voles also experienced a shift in population dynamics on supplemented plots, from typical 3- to 4-year periodicity to an annual cycle. Indeed, they occupy the same ecological niche in northern prairies as that of *S. hispidus* in the southern tallgrass, as *Microtus* is characterized by an herbivorous diet, a requirement for dense vegetative cover, an opportunistic response to habitat conditions, and highly cyclical population dynamics (Birney et al. 1976; Grant et al. 1977; Batzli and Cole 1979). Cole and Batzli (1979) found higher densities, better reproduction and survival, and higher body masses for *M. ochrogaster* in alfalfa fields than in bluegrass or native prairie habitats. Because nitrification of the soil causes plant communities to shift toward nitrogen-responsive species (Tilman 1987; Vitousek 1994), it is reasonable to expect the same responses to habitat alterations within cotton rat populations as in those of their northern analogs.

It is also possible that the other species in my study may have significant effects on nitrogen cycling in supplemented plots. Whereas small mammals alter decomposer and producer communities through activities such as soil translocation, excretion, and herbivory, they also alter dynamics of other taxa (particularly arthropods) through direct predation (Grant and French 1980). Animals that cache nuts and seeds also may aid plant propagation, as some food items undoubtedly escape consumption and go on to germinate (Gessaman and MacMahon 1984). Both species of *Peromyscus* can consume large quantities of insects and gather food items in caches (Grant et al. 1977; Lackey et al. 1985; Sealander and Heidt 1990; Wilson and Ruff 1999; McAdam and Millar 1999). Given their granivorous diet, it also is possible that harvest mice may accumulate nuts

and seeds and likewise affect plant reproduction, although they are not as responsive to habitat type as *S. hispidus* in terms of movements and demography (Cameron and Kincaid 1982).

Studies in several habitat types (e.g., shortgrass prairie, tallgrass prairie, oak woodland) in widespread geographic locations (e.g., Europe, North America, equatorial regions) have shown consistent changes in plant community dynamics due to excess environmental nitrogen (Tilman 1987; Carson and Barrett 1988; Vitousek 1994; Fenn et al. 1998). I predict that similar alterations to small-mammal assemblages will occur concomitantly. Opportunistic species such as *S. hispidus*, with life histories that involve reproductive and physiological flexibility to changing nutrient availability (Lochmiller et al. 2000), will dominate their respective habitats as environmental nitrogen increases. The end result will be a shift in community composition, a decrease in species diversity, and an increase in overall biomass (Grant et al. 1977; Vitousek 1994). The ability of small mammals to contribute substantially to rates of nitrogen flux, along with the general increase in biomass with high soil nitrogen, amplify the rate of nitrogen cycling.

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APPENDIX A

Various researchers have reported changes in gastrointestinal morphology in response to physiological state (Derting 1996; Thompson and Drobney 1996; Derting and Austin 1998), season (Derting and Noakes 1995; Novoa et al. 1996), energy demand (Hammond and Wunder 1991; Derting and Bogue 1993), diet (Hammond and Wunder 1991; Corp et al. 1997) or nutritional restriction, including energy and protein (Lochmiller et al. 1989; Lochmiller et al. 1992; Geluso and Hayes 1999). I analyzed several characteristics of gross gastrointestinal morphology in postweanling juveniles from growth trials to determine the effect of variable nitrogen in postweaning and maternal diets on an individual's capacity to carry out digestive processes.

Materials and Methods

At 44 days postweaning (62 days of age for rats, 65 days for mice), each animal was weighed and put under general anesthesia with Metofane (methoxyflurane, Mallinckrodt Veterinary, Inc., Mundeleine, Illinois) to immobilize them for body composition analysis using the Total Body Electrical Conductivity (TOBEC) technique (Bachman 1994; Buck and Barnes 1999; Unangst and Wunder 2001; results of body composition analyses will not be discussed in this thesis). Body composition analysis was performed at the same time of day (approximately 1900 CST) for all animals. After analysis, animals were placed back into their plastic cages (with the same experimental rations they had been receiving) and transported to the Zoology Department in the Life Sciences West building at Oklahoma State University, where immunological assays and gastrointestinal morphology analyses were carried out.

At 0800 CST on the morning after transport from Laboratory Animal Resources to Life Sciences West, weanlings of both species underwent a preliminary procedure as part of a set of immunological assays (results of immune analyses will not be discussed in this thesis). In random order, they were anesthetized with Metofane and injected with phytohemagglutinin (PHA) diluted in phosphate-buffered saline, which was designed to elicit a localized cell-mediated immune response to be measured with a skin-fold test (these data are not reported in this text). After injection, animals were returned to their cages for 24 hours.

Starting at 0800 CST the next day, animals were again anesthetized in random order, morphometric measurements were taken, and blood samples were collected from the retro-orbital sinus (for immune assays). Animals were euthanized by cervical dislocation and subjected to necropsy. The peritoneal cavity was opened and the liver, stomach, small intestine, cecum, and large intestine were removed and separated from each other. The stomach was cut from the small intestine at the pyloric sphincter, and remains of the esophagus were removed at the cardiac sphincter. The distal small intestine was clipped at the ileocecal junction, and the cecum was separated from the large intestine at the same point. The distal large intestine was cut as close as possible to the anus within the abdominal cavity.

After removal, all organs were placed in phosphate-buffered saline when not being handled. Each organ was trimmed of fat and mesentery, laid flat on a bed of distilled water without stretching, and its length was measured to ± 1 mm. Organs were opened lengthwise and cleaned of contents by flushing with distilled water, then blotted and weighed to ± 0.1 mg. Wet liver mass included the full gall bladder. After obtaining

wet organ masses, all organs were placed on a pre-weighed piece of aluminum and dried at 60°C for at least 48 hours. Organs were removed from the drying oven and cooled in a dessicator before re-weighing to determine dry mass.

All data from juvenile necropsies were corrected for the confounding influences of body mass by regressing the logarithm of each organ parameter (length, wet mass or dry mass) on log body mass and finding the residual for each data point (Barton and Houston 1994; Hilton et al. 1999). Comparisons of the residual variation around the resulting regression lines were used for all subsequent analyses. To test the response of digestive organs to dietary nitrogen, within-species data were analyzed using the same multiple regression method, with the same set of potential independent variables and stepwise model-building procedure, as for growth parameters. Regressions were tested for sex effects using indicator variables, and separate regressions were carried out for males and females where necessary. Interspecies comparisons of necropsy data were accomplished through 2x2 factorial ANOVA (species by sex; PROC MIXED) of organ residuals with a split-plot design to test for within-species sex differences.

Results

Small intestine length (n = 128, P = 0.01), large intestine length (n = 131, $P <$ 0.001), dry stomach mass (n = 126, P = 0.01), and dry large intestine mass (n = 117, P = 0.01) varied by sex across species. Several variables were affected by the interaction of species and sex. Wet liver mass varied by sex for *R. fulvescens* only ($P < 0.001$), wet stomach and large intestine mass varied by sex for *S. hispidus* only ($P < 0.001$ and P = 0.01, respectively), and dry liver mass varied by sex for *R. fulvescens* only (P = 0.01). In all cases but 2, females had longer or more massive digestive organs. However, wet and

dry liver mass for male *R. fulvescens* was greater. There was a marginal species difference in dry liver mass for males ($P = 0.05$), but no other species differences were detected for any of the residual organ parameters measured ($P \geq 0.09$)

Regression analysis of *S. hispidus* residual organ lengths and masses using indicator variables showed sex differences in organ responses to dietary nitrogen for large intestine length ($n = 65$, $P = 0.01$), wet stomach mass ($n = 61$, $P = 0.02$), wet large intestine mass ($n = 53$, $P = 0.01$, and dry large intestine mass ($n = 53$, $P = 0.05$). Sexes were therefore considered separately for these organs when building multiple regression models.

I failed to find a relationship with any potential independent variable for cotton rat stomach length, dry liver mass, or wet and dry small intestine mass. Residual small intestine length was related to both maternal nitrogen intake (%) and the square of maternal diet ($n = 64$, $P < 0.001$, $R^2 = 0.23$; Table A1, Equation 56; Table A2). Residual cecum length showed a relationship with postweaning nitrogen intake (%), maternal nitrogen intake (%), and the square of maternal diet ($n = 65$, $P < 0.001$, $R^2 = 0.30$; Table A1, Equation 57; Table A2). Mass-corrected large intestine length for males responded to the square of postweaning nitrogen intake (%) ($n = 27$, $P = 0.03$, $r^2 = 0.18$; Table A1, Equation 58; Table A2), and residual large intestine length for females was related to the interaction between maternal and postweaning diet ($n = 38$, $P = 0.007$, $r^2 = 0.19$; Table A1, Equation 59; Table A2).

Analysis of residual wet liver mass for *S. hispidus* showed a relationship with % maternal nitrogen intake and the square of postweaning dietary nitrogen (%; $n = 61$, $P =$

Table A1.—Equations for predicting parameters of gastrointestinal organs for *S. hispidus* and *R. fulvescens* under variable % maternal and post-weaning dietary nitrogen.

Equation #	Species	Equation	Parameters of regression model		
			n	p-value	r^2
56	*S. hispidus*	residual small intestine length = 0.14 – 0.1393 * (% maternal N intake) + 0.0297 * (% maternal N intake)^2	64	< 0.001	0.23
57	*S. hispidus*	residual cecum length = 0.09 + 0.0214 * (% post-weaning N intake) – 0.1350 * (% maternal N intake) + 0.0279 * (% maternal N intake)^2	65	< 0.001	0.30
58	*S. hispidus*	residual male large intestine length = -0.03 + 0.0029 * (% post-weaning N intake)^2	27	0.03	0.18
59	*S. hispidus*	residual female large intestine length = -0.03 + 0.0058 * (% maternal N intake) * (% post-weaning N intake)	38	0.007	0.19
60	*S. hispidus*	residual wet liver mass = 0.01 – 0.0128 * (% maternal N intake) + 0.0040 * (% post-weaning N intake)^2	61	0.004	0.17
61	*S. hispidus*	residual female wet stomach mass = -0.06 + 0.0319 * (% post-weaning N intake)	36	0.003	0.24
62	*S. hispidus*	residual wet cecum mass = -0.05 + 0.0086 * (% post-weaning N intake)^2	53	0.003	0.17
63	*S. hispidus*	residual male wet large intestine mass = -0.13 + 0.0094 * (% post-weaning N intake)^2 + 0.0053 * (% maternal N intake)^2	21	0.004	0.45

Table A1 (continued).

Equation #	Species	Equation	Parameters of regression model		
			n	p-value	r^2
64	*S. hispidus*	residual female wet large intestine mass = -0.05 + 0.0118 * (% post-weaning N intake) * (% maternal N intake)	32	0.004	0.24
65	*S. hispidus*	residual dry stomach mass = -0.04 + 0.0062 * (% post-weaning N intake)^2	61	0.02	0.09
66	*S. hispidus*	residual dry cecum mass = -0.10 + 0.0411 * (% post-weaning N intake)	53	0.01	0.12
67	*S. hispidus*	residual male dry large intestine mass = -0.07 + 0.0074 * (% post-weaning N intake) * (% maternal N intake)	21	0.11	0.13
68	*S. hispidus*	residual female dry large intestine mass = -0.06 + 0.0130 * (% post-weaning N intake) * (% maternal N intake)	32	0.006	0.23
69	*R. fulvescens*	residual stomach length = -0.03 + 0.0046 * (% post-weaning N intake) * (% maternal N intake)	66	0.03	0.08
70	*R. fulvescens*	residual small intestine length = -0.04 + 0.0053 * (% post-weaning N intake)^2	64	0.02	0.09
71	*R. fulvescens*	residual cecum length = -0.05 + 0.0076 * (% post-weaning N intake)^2	67	< 0.001	0 19
72	*R. fulvescens*	residual large intestine length = -0.02 + 0.0030 * (% post-weaning N intake)^2	66	0.05	0.06

Table A1 (concluded).

Equation #	Species	Equation	Parameters of regression model		
			n	p-value	r^2
73	*R. fulvescens*	residual wet liver mass = -0.04 + 0.0057 * (% post-weaning N intake)^2	65	< 0.001	0.18
74	*R. fulvescens*	residual wet stomach mass = -0.04 + 0.0052 * (% post-weaning N intake)^2	65	< 0.001	0.23
75	*R. fulvescens*	residual wet cecum mass = -0.44 + 0.0582 * (% post-weaning N intake) + 0.2847 * (% maternal N intake) – 0.0633 * (% maternal N intake)^2	65	0.002	0.22
76	*R. fulvescens*	residual wet large intestine mass = -0.09 + 0.0120 * (% post-weaning N intake)^2	35	0.005	0.21
77	*R. fulvescens*	residual dry liver mass = -0.04 + 0.0056 * (% post-weaning N intake)^2	65	0.001	0.15
78	*R. fulvescens*	residual dry stomach mass = -0.05 + 0.0078 * (% post-weaning N intake) * (% maternal N intake)	65	0.001	0.15
79	*R. fulvescens*	residual dry cecum mass = -0.16 * 0.0608 * (% post-weaning N intake)	65	0.005	0.12
80	*R. fulvescens*	residual dry large intestine mass = 0.04 – 0.0313 * (% maternal N intake) + 0.0058 * (% post-weaning N intake)^2	64	0.08	0.08

Table A2.—Results of multiple regression analysis of the effects of % maternal and post-weaning nitrogen intake on gross gastrointestinal morphology in juvenile *S. hispidus* and *R. fulvescens*. Analyses are based on mass-corrected residuals of specified organ parameters. Significant independent variables are indicated (+ or – indicates direction of effect; + = $0.15 > P > 0.05$; + + = $0.05 > P > 0.01$; + + + = $P < 0.01$).

Dependent variable	Potential independent variables				
	% maternal N intake	(% maternal N intake)^2	% post-weaning N intake	(% post-weaning N intake)^2	(% maternal N intake) x (% post-weaning N intake)
S. hispidus					
Small intestine length	– – –	+ + +			
Cecum length	– – –	+ + +	+ + +		
Large intestine length					
Males				+ +	
Females					+ + +
Wet liver mass	–			+ +	
Wet stomach mass					
Females			+ + +		
Wet cecum mass				+ + +	

Table A2 (continued).

Dependent variable	Potential independent variables				
	% maternal N intake	(% maternal N intake)^2	% post-weaning N intake	(% post-weaning N intake)^2	(% maternal N intake) x (% post-weaning N intake)
S. hispidus					
Wet large intestine mass					
Males		+		+++	
Females					+++
Dry stomach mass				++	
Dry cecum mass			+++		
Dry large intestine mass					
Males					+
Females					+++

Table A2 (continued).

Dependent variable	Potential independent variables				
	% maternal N intake	(% maternal N intake)^2	% post-weaning N intake	(% post-weaning N intake)^2	(% maternal N intake) x (% post-weaning N intake)
R. fulvescens					
Stomach length					+ +
Small intestine length				+ +	
Cecum length				+ + +	
Large intestine length				+	
Wet liver mass				+ + +	
Wet stomach mass				+ + +	
Wet cecum mass	+ +	– –	+ + +		
Wet large intestine mass					
Males				+ + +	
Dry liver mass				+ + +	

Table A2 (concluded).

Dependent variable	Potential independent variables				
	% maternal N intake	(% maternal N intake)^2	% post-weaning N intake	(% post-weaning N intake)^2	(% maternal N intake) x (% post-weaning N intake)
R. fulvescens					
Dry stomach mass					+ + +
Dry cecum mass			+ + +		
Dry large intestine mass	–			+	

0.004, $R^2 = 0.17$; Table A1, Equation 60; Table A2). After correction for body mass, male wet stomach mass did not respond to any independent variable; however wet stomach mass for females was linearly related to postweaning nitrogen intake (%) (n = 36, $P = 0.003$, $r^2 = 0.24$; Table A1, Equation 61; Table A2). Residual wet cecum mass responded to the square of postweaning nitrogen intake (%) ($n = 53$, $P = 0.003$, $r^2 = 0.17$; Table A1, Equation 62; Table A2). Wet large intestine mass for males responded to the square of postweaning diet, as well as the square of maternal diet ($n = 21$, $P = 0.004$, $R^2 = 0.45$; Table A1, Equation 63; Table A2), and the same variable for females was related to the interaction between maternal and postweaning nitrogen intake (%) ($n = 32$, $P = 0.004$, $r^2 = 0.24$; Table A1, Equation 64; Table A2).

Mass-corrected dry stomach mass for cotton rats showed a relationship with the square of postweaning nitrogen intake (%) ($n = 61$, $P = 0.02$, $r^2 = 0.09$; Table A1, Equation 65; Table A2). Residual dry cecum mass was linearly related to postweaning nitrogen intake (%) ($n = 53$, $P = 0.01$, $r^2 = 0.12$; Table A1, Equation 66; Table A2). Residual dry large intestine mass for males was only weakly related to the interaction between maternal and postweaning diet ($n = 21$, $P = 0.11$, $r^2 = 0.13$; Table A1, Equation 67; Table A2), although dry large intestine mass for females showed a significant relationship with the same maternal-postweaning diet interaction ($n = 32$, $P = 0.006$, $r^2 = 0.23$; Table A1, Equation 68; Table A2).

The same set of analyses for *R. fulvescens* indicated significant sex effects for the relationship between wet large intestine mass and dietary nitrogen; this was the only multiple regression model where males and females needed to be considered separately.

I also failed to find a relationship between wet or dry small intestine mass and any independent variable.

Residual stomach length responded to the interaction between maternal and postweaning nitrogen intake (%) ($n = 66$, $P = 0.03$, $r^2 = 0.08$; Table A1, Equation 69; Table A2). Mass-corrected data for small intestine length, cecum length, large intestine length, wet liver mass, and wet stomach mass were all related to the square of postweaning nitrogen intake (%) (small intestine length: $n = 64$, $P = 0.02$, $r^2 = 0.09$; cecum length: $n = 67$, $P < 0.001$, $r^2 = 0.19$; large intestine length: $n = 66$, $P = 0.05$, $r^2 = 0.06$; wet liver mass: $n = 65$, $P < 0.001$, $r^2 = 0.18$; wet stomach mass: $n = 65$, $P < 0.001$, $r^2 = 0.23$; Table A1, Equations 70-74; Table A2). Residual wet cecum mass showed a relationship with postweaning dietary nitrogen (%), maternal nitrogen intake (%), and the square of maternal diet ($n = 65$, $P = 0.002$, $R^2 = 0.22$; Table A1, Equation 75; Table A2). I did not find a relationship between wet large intestine mass for females and any potential independent variable; however male wet large intestine mass, as well as dry liver mass for both sexes was related to the square of postweaning diet (%) (male wet large intestine mass: $n = 35$, $P = 0.005$, $r^2 = 0.21$; dry liver mass: $n = 65$, $P = 0.001$, $r^2 = 0.15$; Table A1, Equations 76-77; Table A2). Residual dry stomach mass responded to the interaction between maternal and postweaning diet ($n = 65$, $P = 0.001$, $r^2 = 0.15$; Table A1, Equation 78; Table A2). Mass-corrected dry cecum mass showed a linear relationship with postweaning dietary nitrogen (%) ($n = 65$, $P = 0.005$, $r^2 = 0.12$; Table A1, Equation 79; Table A2), and residual dry large intestine mass was weakly related to

both maternal nitrogen intake (%) and the square of % postweaning dietary nitrogen (n = 64, $P = 0.08$, $r^2 = 0.08$; Table A1, Equation 80; Table A2).

Discussion

Differences in gastrointestinal morphology of mammals have been observed in response to a number of factors. Wood mice from distinct populations, each with differing food availability and diet type, showed differences in intestine length (Corp et al. 1997), such that woodland granivores had longer intestines than desert insectivores. Prairie voles consuming high-fiber diets in the laboratory developed larger hindguts, in mass as well as length (Hammond and Wunder 1991). Increases in energy demand, such as cold acclimation, lactation, induced changes in metabolic rate, or molt, cause corresponding increases in food intake rates and accretion of tissue in the digestive tract (Hammond and Wunder 1991; Derting and Bogue 1993; Derting and Noakes 1995; Derting 1996; Novoa et al. 1996; Thompson and Drobney 1996; Derting and Austin 1998).

Nutritional limitation also has induced changes in digestive morphology, as cell hypertrophy or hyperplasia occurs in an attempt to increase assimilation efficiency. Protein and energy restriction in female collared peccaries resulted in larger relative stomach mass (Lochmiller et al. 1989). Low-energy, high-fiber diets caused a significant increase in dry mass of all gastrointestinal organs, including the liver, in European starlings (Geluso and Hayes 1999). Protein-restricted juvenile and sub-adult cotton rats experienced an accretion in gut-associated lymphoid tissue (Lochmiller et al. 1992). Juvenile cotton rats fed a protein-deficient (4% crude protein) monocot diet showed higher liver mass than those fed an adequate (11% crude protein) dicot diet (Cameron and Eshelman 1996). Because the first weeks of life are a crucial phase in the development

of many organ systems, any compromise in maternal plane of nutrition may have serious consequences. Virgl and Messier (1992) found that the digestive tract in nursling muskrats grows very rapidly, such that by weaning 75% of the adult intestine is already formed. Accordingly, there was a high potential for alterations of gastrointestinal tract morphometry under the conditions of my study, as a result of either the pre-weaning nutritional environment or the postweaning level of nitrogen intake.

The lack of a relationship between gastrointestinal morphometry and maternal or postweaning nitrogen intake for some of the organs measured, particularly for *S. hispidus* dry liver mass, is difficult to interpret. The lack of a relationship between stomach length and dietary nitrogen for *S. hispidus* is likely the result of the high degree of variation I observed in stomach fill, particularly in the fundus. Because of the role of this portion of the stomach in secondary fermentation of ingested cecal pellets (Kenagy and Hoyt 1980), it exhibits a large capacity for distention.

It is particularly noteworthy that I found no relationship between any measure of small intestine mass and dietary nitrogen for either species. It is possible that the tissues of the small intestine are too plastic to still show the influence of long-term nutritional conditions. However, I suspect that the delicate nature of the tissues resulted in unintentional damage during the cleaning process after necropsy. As a consequence, a high degree of variation was introduced into my data, obscuring any significant relationships between small intestine parameters and maternal or postweaning diet. Nevertheless, I found significant relationships between small intestine length and dietary nitrogen for both species. This relationship may be used as a surrogate to describe the response of this organ to the nutritional environment.

Overall, I found positive relationships between digestive organ parameters and postweaning nitrogen intake, and negative relationships between organ measurements and maternal nitrogen intake. In other words, offspring of mothers on low-nitrogen diets tended to have longer or larger gastrointestinal organs, whereas weanlings receiving low-nitrogen diets had shorter, smaller guts. The trend with respect to maternal nutrition is consistent with previous studies, which found a general accretion of gastrointestinal tissue in response to poor nutritional environments (Lochmiller et al. 1989, 1992; Cameron and Eshelman 1996; Geluso and Hayes 1999). I suggest that offspring of low-nitrogen litters grow more gut tissue in anticipation of poor nutrient availability upon weaning, when they would then be capable of higher digestive efficiencies. The observations based on postweaning diet are likely a direct effect of resource availability, as individuals on low-nitrogen diets simply do not have sufficient nitrogen to build and maintain digestive organs.

My analysis of digestive organ dimensions underscored the important role held by maternal nutritional conditions and those experienced postweaning in the formation of key organ systems. This was especially apparent in *S. hispidus*, as most digestive organs for this species responded to some combination of maternal and postweaning influences. Overall, the trend in digestive organ parameters mirrors that shown in regression analysis of growth traits, with a weaker maternal influence for *R. fulvescens*. In light of the previously mentioned variation in nursling development in cotton rats, as well as the importance of the pre-weaning period to the ontogeny of digestive organs (Virgl and Messier 1992), it is not surprising that the organ systems of neonatal cotton rats benefited from a larger nutritional input.

VITA

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